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DETERMINING HEAT PRODUCTION OF BLACK SOLDERI FLY LARVAE, *HERMETIA ILLUCENS*, TO DESIGN REARING STRUCTURES AT LIVESTOCK FACILITIES

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DETERMINING HEAT PRODUCTION OF BLACK SOLDERI FLY LARVAE, *HERMETIA ILLUCENS*, TO DESIGN REARING STRUCTURES AT LIVESTOCK FACILITIES

THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biosystems and Agricultural Engineering in the Colleges of Agriculture and Engineering at the University of Kentucky

By

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Lexington, Kentucky

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Lexington, Kentucky

2018

ABSTRACT OF THESIS

DETERMINING HEAT PRODUCTION OF BLACK SOLDERI FLY LARVAE, *HERMETIA ILLUCENS*, TO DESIGN REARING STRUCTURES AT LIVESTOCK FACILITIES

Due to their small size and ectothermic biology, the heat production of insects and insect larvae is hard to quantify. However, knowing the amount of heat production, as well as ammonia production of insects may be beneficial for commercial production of valuable insect species. Black soldier fly larvae (BSFL) are of interest in the agricultural industry because they quickly consume organic waste and have high amounts of protein and fat in their bodies. It has been proposed that BSFL be used to manage livestock waste, while serving as a high-protein feed source for livestock animals. To efficiently rear BSFL, it is necessary to design rearing facilities, which maintain optimal conditions for the larvae. To design such a facility, it is necessary to know the amount of heat and ammonia that BSFL produce.

A gradient calorimeter was used to measure the heat and ammonia production rates of black soldier fly larvae. The study determined that BSFL heat production changes significantly with the age and weight of the larvae. Aggregations produce the most total heat when larvae are older and larger. The study also found that larvae produce less heat per individual and per gram of body weight as they grow. Larvae also produce significantly different amounts of heat depending on the size of the groups they are in, and do not produce consistent amounts of heat per individual or per gram of body weight, even if maintained at a consistent population density. Larvae in group sizes of 100, 300, and 500 produced an average and standard deviation of 0.00107±0.000295, 0.00067±0.00014, and 0.00049±0.00020 W/larva, respectively. Likewise, larvae in groups of 100, 300 and 500 produced an average of 0.01826±0.00010, 0.01023±0.00565, and 0.00575±0.00371 W/g, respectively. The differences in heat produced per individual and per gram is troublesome when trying to estimate a total heat production for large populations.

The largest heat production rate observed in this study was 0.407 W, and was produced by a group of 500 BSFL. Frass analysis indicated that between 4.80 and 7.79 lbs of ammoniacal-nitrogen is emitted for every ton of frass produced. These data could be used to estimate the total heat and ammonia produced from a larger population of BSFL being reared inside a closed facility, allowing engineers to design HVAC systems to keep the larvae at their optimal growing condition year-round. Placing BSFL rearing accommodations at livestock facilities could be beneficial to livestock, poultry, and fishery producers, because BSFL can be used to dispose of animal wastes and are also a good source of protein-rich animal feed.

Keywords. Calorimetry, Gradient Calorimeter, Seebeck Effect, Thermocouple

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December 12, 2018
Date

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Introduction

Black soldier flies, *Hermetia illucens,* are a common fly species native to North America. Black soldier fly larvae (BSFL) are known for quickly consuming many types of organic wastes, making them a viable option for waste management (Bradley and Sheppard, 1983; Denier et al., 2011; Newton et al., 2005). Due to their high protein and fat content, BSFL have also been suggested as an alternative feed source for livestock and fish (Denier et al., 2011; Newton et al., 2005; Sheppard et al, 1994; St-Hillaire et al., 2007). Since current feed production relies on the extraction of some proteins and fats from fish, discovering an alternative protein source for animal feed could reduce pressure on wild fish populations (Alder et al., 2008; Denier et al., 2011).

Therefore, implementing BSFL as an on-site waste management practice at livestock and fishery facilities can serve multiple purposes. First, animal wastes such as manure, or fish offal, could be fed to BSFL, providing easy on-site waste management. Second, once BSFL reach maturity and cease their consumption of wastes, they could be processed on-site and used as a supplemental, high-protein feed source for animals at the facility. As a result, an on-site BSFL-rearing facility would provide livestock operations with a local and sustainable source of waste management and animal feed. Additionally, BSFL may reduce the risk of both humans and animals being exposed to disease, as they have been shown to suppress pathogens like *Escherichia coli* and *Salmonella* spp. (Lalander et al., 2013; Erickson et al., 2004; Liu et al., 2008), as well as populations of disease transmitting flies (Sheppard et al., 1983).

However, efficiently raising BSFL near livestock facilities may not be straightforward. BSFL are very sensitive to their environment. Their growth and development are dependent upon environmental conditions – particularly temperature – in addition to food availability (Denier et al., 2009; Park, 2016; Sheppard et al., 2002; Tomberlin et al., 2009). The ideal temperature for BSFL growth is 27° C (Park, 2016; Tomberlin, 2009). Thus, at this temperature BSFL grow the fastest, would remove more waste from the livestock facility, and would provide

a more readily available food source. Consequently, an optimally efficient BSFL rearing facility would need to be kept at 27° C year-round; meaning the facility would need to be indoors for precision environmental control.

Black soldier fly larvae produce heat through metabolism and movement. When moving, friction with their environment – which usually includes friction from rubbing against other larvae – can be an important source of a larva's heat production. Unfortunately, the amount of heat produced by BSFL is not known. If an efficient rearing facility is to be designed, the heat generated by BSFL needs to be accounted for so that proper amounts of supplemental heat and cooling can be added to maintain ideal temperatures. Additionally, BSFL produce ammonia in their waste (Green et al., 2012), so the amount of gaseous ammonia produced needs to be known to ensure the facility receives enough fresh air to keep the structure healthy for BSFL and human occupants.

Ultimately, the goal of this project was to acquire heat and ammonia production data for black soldier fly larvae. Ideally, others will be able to use this information later to design rearing facilities for the larvae.

Objective 1 – Gradient Calorimeter

An organism's movement and metabolism inevitably produces heat, which can be measured using calorimetry. Gradient calorimetry is a form of direct calorimetry and accounts for all of an organism's heat production. Gradient calorimetry uses thermocouples to measure heat flux across a gradient layer. This project used a gradient calorimeter to study the heat production of BSFL. Therefore, the first objective of the study was to design and build a gradient calorimeter.

Objective 2 – Measure Heat and Ammonia Production

The gradient calorimeter creates an enclosed space with no, or limited, air exchange with the outside surroundings. Therefore, virtually all of the heat produced by the BSFL within the space was measured by the calorimeter. Since the calorimeter was comprised of an enclosed area, an ammonia sensor was used to measure changes in ammonia concentration in the space over time. The calorimeter

was thus used to measure both the heat production and ammonia production of BSFL at the same time. Additionally, frass samples from the larvae were collected throughout the experiments and were sent to a manure and soil testing laboratory to be analyzed for ammonia and total nitrogen content.

End Goal – Provide Heat and Ammonia Data for Designing Rearing Facilities

Ideally, the data collected in this study will be useful for approximating how much heat and ammonia would be produced by a large BSFL population kept in a specified volume of space. Having an accurate estimate of how much heat and ammonia the larvae will produce will allow engineers to determine how much heating, cooling, and ventilation will be needed within a space to keep the larvae at their ideal conditions throughout the year, taking into account outside conditions for a given location. Efficient BSFL rearing facilities could then be implemented onsite at livestock, poultry, and fishery operations to serve as a local waste management and a high-protein feed source.

Literature Review

Objective 1 – Gradient Calorimeter

What is Calorimetry Used For?

Calorimetry is a technique often used to measure the energy content of a substance. It can also be used to determine the metabolism rate and heat production of an organism. For large creatures, such as human beings and livestock animals, indirect calorimetry is a method typically used to estimate the amount of heat produced. Indirect calorimetry measures the rate of oxygen consumed and the amount of carbon dioxide emitted by an animal and uses this information to calculate the amount of heat being produced (Head et al., 1984). Equation 1, which was empirically derived from measured data, shows the general form of the equation used to determine the heat production rate of an organism (Brouwer, 1965; Bridges et al., 2009). Equation 1 takes into account the amounts of methane and nitrogen excreted in addition to the amount of oxygen consumed and the carbon dioxide emitted, because methane and nitrogen production can be significantly correlated to the heat production of some animals, such as ruminants. However, for animals that do not produce notable amounts of methane or nitrogen, Equation 2, which is a simplified version of Equation 1, can be used to estimate the heat production of an organism.

$$HP = 16.18O_2 + 5.02CO_2 - 2.17CH_4 - 5.99N \quad (1)$$

Where: HP = metabolic heat production rate (W)
O_2 = oxygen consumption rate (mL/s)
CO_2 = carbon dioxide production rate (mL/s)
CH_4 = methane production rate (mL/s)
N = nitrogen excretion rate (g/s)

$$HP = 16.18O_2 + 5.02CO_2 \quad (2)$$

While convenient for use on large animals with easily measureable gas consumptions and emissions, indirect calorimetry can be more challenging when attempting to measure the small quantities of gases consumed and produced by insects. Therefore, this study chose to use direct calorimetry to measure the heat

production rate of these organisms. The most straightforward and accurate form of direct calorimetry is gradient calorimetry (Lighton, 2008). This type of calorimetry isolates the study organism from the outside environment and directly measures the amount of heat produced using a series of connected thermopiles. Although gradient calorimeters may require complicated designs, they are easy to use and calibrate. These types of calorimeters are typically constructed in the form of a box, so that the study organism can be completely enclosed by the device. Consequently, all of the heat produced by the animal will transfer through the walls and be measured by the sensors on the interior of the device. Therefore, gradient calorimetry is simpler and potentially more accurate, because all of the heat produced by the animal is measured directly, thus eliminating potential error from gas measurements.

Principles of Gradient Calorimetry

Gradient calorimetry works by employing the Seebeck Effect. The Seebeck Effect states that if two different conductive metals are in contact with one another, then those metals will produce a voltage when exposed to a temperature gradient (Lighton, 2008). This is the principle that makes the use of thermocouples possible, and thermocouples are the tools used to measure heat production in a gradient calorimeter.

As stated above, gradient calorimeters isolate the study organism from the outside environment. Gradient calorimeters create a thermally conductive gradient layer to isolate these organisms. Thermocouples are woven back and forth across the gradient layer, thereby sensing temperatures on both the inside and outside of the isolation layer. The thermocouples on the inside of the gradient layer sense the temperature of the inside of the chamber while the thermocouples on the outside sense the outer temperature. Typically, an additional layer of conductive material, such as metal, is placed adjacent to the outer surface of the gradient layer, flush with the outer thermocouples. This outer shell is kept at a constant, cooler temperature and acts as a heat sink for the heat produced by the organism in the isolation

chamber. As heat generated by the organism on the inside of the chamber travels outwards through the gradient layer to the cooler heat sink, a temperature gradient is created across the thermocouples. This temperature gradient causes the thermocouples to produce a voltage which is correlated to the amount heat passing through the gradient layer – the more heat produced by the organism, the higher the voltage produced by the thermocouples.

To simplify measurements, all of the thermocouples in the gradient layer should be connected in series as they wind back and forth from one side of the gradient layer to the other. Connecting many thermocouples in series like this creates what is referred to as a thermopile. Thermopiles provide two advantages for the construction of a gradient calorimeter. First, a large thermopile covering each surface of the calorimeter ensures that all of the heat produced by the study organism will be accounted for in the voltage measurement. Second, connecting all of the thermocouples and thermopiles in series will result in a single output voltage. The output voltage can then be read using a thermocouple reader or a voltage meter.

Once the gradient calorimeter is constructed, calibration of the device is simple. One only needs to apply a known amount of heat to the inside of the calorimeter, while recording the voltage output of the thermopiles. When heat is applied inside the isolation chamber, the voltage output will steadily rise until equilibrium is reached, at which point the amount of heat being produced inside the chamber equates to the amount of heat being displaced through the outer shell and into the environment. At this equilibrium point, the voltage output should stop increasing and maintain a constant value. The constant voltage can then be paired with the known heat that was applied for that calibration run. This procedure should be repeated for a range of heat exposures on the inside of the box. After all points are collected, the amount of heat inside the chamber can be plotted against the voltage produced by the thermopiles to yield a linear relationship between voltage output and interior heat production. Using this relationship, the amount of heat produced by an organism inside the calorimeter can be calculated from the voltage output produced by the device.

While not common, gradient calorimeters have been used to measure the heat production and metabolism rates of some large animals, including humans (Seale et al., 1991). Seale's study realized the importance of not allowing the study organisms being studied to make physical contact with the gradient layer, or else risk inconsistent and inaccurate heat production measurements. Seale also detailed the importance of connecting all thermopiles in series, in order to yield a single voltage reading.

For this study, a much smaller calorimeter was used, similar to one constructed at the Department of Physiology and Biophysics of Indiana University (Lamprecht et al., 1998). Lamprecht (1998) designed a box out of 9-mm aluminum walls with interior dimensions of 150*150*150-mm. The calorimeter included two ports in the sides of the container to provide airflow into and out of the chamber. The chamber was also insulated on the outside of the aluminum walls with an 8-mm thick layer of Styrofoam. They used a 1mm thick gradient layer inside of the box and equally spaced thermocouples throughout the gradient layer to evenly cover all six sides of the box. A total of 1,575 thermocouples made up the thermopile in their calorimeter, which had a total resistance of 27.7 ohms. During data collection, the calorimeter was kept inside of a thermostatic cabinet, which maintained a desired outer-shell temperature.

Studies that have used calorimetry to examine different species of insects found that the insects generally produced more heat when moving and during digestion (Kurtti et al., 1978). Furthermore, experiments on larvae of similar size to BSFL found that movement of the larvae contributed from 0.9% to 1.24% of total heat production (Harak et al., 1996). Therefore to get an accurate idea of how much heat the BSFL produced during regular behavior, the calorimeter in this study was large enough for the larvae to move freely and feed while under observation.

Objective 2 – Measure Heat and Ammonia Production

To measure the voltage produced by the calorimeter, the thermopile inside was connected to a thermocouple reader. Unfortunately, thermocouple readers available during this project only recorded temperature. That is, the reader received

a voltage reading from the thermopile, and automatically converted that voltage reading to a temperature, depending on the type of thermocouple attached to the reader. However, since the temperature reading ultimately corresponded to the heat production rate produced by the animals, the calorimeter could be calibrated using known heat rates, as described above, and plotting the heat rates against the temperature readouts of the thermocouple reader. The resulting relationship allowed for the heat production of BSFL larvae to be calculated from the temperature readout of the thermocouple reader.

Ammonia production was measured at the same time as heat production. The calorimeter included two ports for air to travel in and out of the holding chamber, similar to the 1998 Lamprecht design. Therefore, ammonia concentration in the outgoing air could be measured and used to determine the amount of ammonia being generated by the BSFL in a given amount of time. Studies with green bottle fly larvae found that ammonia production was much higher during larval stages than during other stages of their lifecycle (Brown, 1938). Knowing that BSFL also produce ammonia in their waste (Green et al., 2012), we hypothesized that that BSFL would produce high levels ammonia as well during their larval stage, even though it has been determined that different insect species excrete different amounts of ammonia in their waste (Brown, 1938; Oonincx et al., 2010).

Furthermore, at least one study with other fly larvae suggested that the amount of heat produced by the larvae may vary depending on the size of the group of the larvae (Heaton et al., 2014). By measuring the ambient air temperature immediately outside of different sized aggregates of green bottle flies, Heaton et al. found that ambient air temperatures showed the largest increases around the larger aggregates of larvae (2014). More bodies producing more heat is expected. However, this conclusion does not provide a good method for estimating the heat production of a large group of larvae. This study sought to determine, does the heat production per gram of larvae or per individual larvae also change as the group size changes? We sought to determine this in our study by measuring heat production in aggregate sizes of 100, 300, and 500 larvae. This was an important question, because if it was determined that the amount of heat generated per gram of larvae,

or for each individual larvae, does not change significantly with group size, then it would be much easier to estimate how much heat would be produced in enclosed facilities with varied amounts of larvae inside.

Methods and Materials

Objective 1 – Gradient Calorimeter

Gradient Calorimeter Design

The original concept for this calorimeter design came from a calorimeter constructed at the Department of Physiology and Biophysics at Indiana University (Lamprecht et al., 1998). Similar materials were used in both designs, however, this project constructed a calorimeter a bit larger than that described by Lamprecht.

The outer shell of the calorimeter was built using 0.95 cm (3/8 inch) thick aluminum plating (see Figure 1). Five plates were cut and welded together to form the base and four sides of a cube, with inner dimensions of 20.3 cm long, 20.3 cm wide, and 15.25 cm high (eight inches by eight inches by six inches). The top of the plate was cut to the same size as the base plate, but was not permanently fixed to the rest of the shell. One hole was drilled into each of the four corners of the top, along with each of the corners at the top of the cube walls, so that the top plate could be screwed securely into place with four hex screws. Additionally, two holes were bored into the top plate and fitted with aluminum tubing to act as air ports into and out of the calorimeter chamber. This allowed for airflow into and out of the chamber, in the case that the study organisms could not survive long enough with the air inside the chamber to record data measurements.

Figure 1. The outer aluminum shell of the gradient calorimeter, complete with two airflow ports.

The gradient layer of this calorimeter was constructed using 0.16cm (1/16 inch) thick polycarbonate plastic sheeting (ePlastics Item ID**:** PCCLR0.060AM24X48). Base and top panels of the gradient layer were cut as 19.7 cm by 19.7 cm (7.75 inch by 7.75 inch) sheets, with the top panel having two holes cut into the center to match the air ports in the top of the shell for air passage. Two of the side panels were cut into 19.7 cm by 10.8 cm (7.75 inch by 4.25 inch) panels, and the final two side panels were cut into 16.5 cm by 10.8 cm (6.50 inch by 4.25 inch) plates. All six panels had 0.16 cm (1/16 inch) diameter holes drilled through them in a grid pattern, with 0.3175 cm (1/8 inch) spacing between the centers of each hole. The holes covered all of each panel, to within 0.3175 cm (1/8 inch) from the edge of the panel.

Thermocouple wire (24 gauge, T-type, stranded wire, Omega Engineering, item ID 12TX24SPP) was placed into the grid holes. Before being placed into the grid holes, each thermocouple wire was cut into a one-inch segment and the

insulation was stripped on each end, leaving only a small amount of insulation around the middle of the wire, where the wire passed through the hole. The cut and stripped wires were then placed into the grid holes, alternating between copper (blue) and constantan (red) wires. Once in place, the wires were twisted together to form a continuous series of thermocouple junctions, which wove between each side of the panels. Wires were twisted together to within a 0.3175 cm (1/8 inch) of the polycarbonates panels, and the excess wire was cut off. One long wire was kept at two of the corners of each panel (at the end of the thermocouple strand) to be used later to connect all thermopiles together.

The top and bottom panels contained 30 rows and 30 columns of thermocouple wire, as shown in Figure 2. The top panel had several fewer total junctions due to the holes cut in the middle of the panel for airflow. The long side panels container 16 rows by 30 columns of wire, and the short sides 16 rows by 26 columns. The final apparatus contained approximately 3,500 thermocouple junctions, once losses from the airflow holes were taken into account.

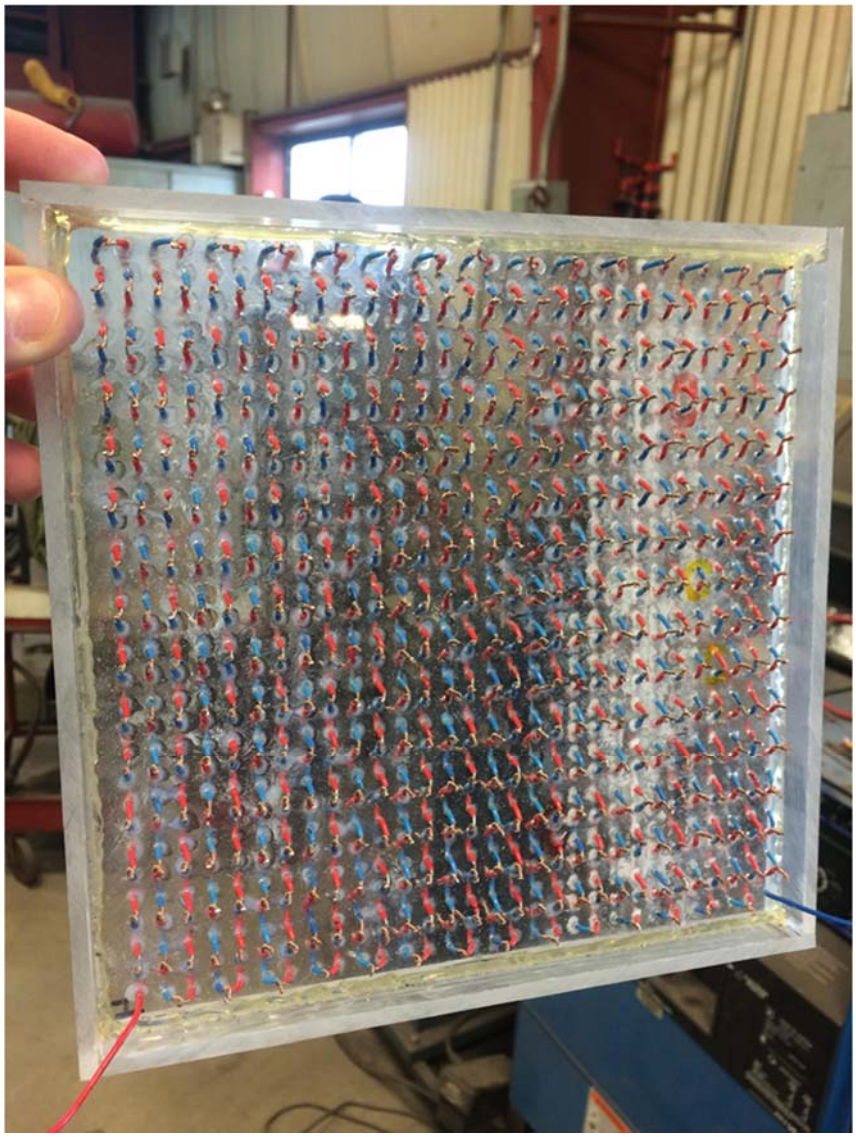

Figure 2. The completed wiring arrangement of the bottom thermocouple, before epoxy was put in place.

Once all wire junctions were in place, each side of each panel was coated in epoxy (Smooth-On EpoxAcast 690), until all wire junctions were submerged. Since the wires were only twisted together and not soldered, this step was taken to secure the thermocouple junctions and protect them from damage. Creating a flat layer of epoxy on each panel also enabled the panels to be laid flush against the aluminum shell to optimize heat transfer through the gradient layer.

To pour the epoxy, frames were built out of polycarbonate. The frames were cut to extended ½ inch on either side of the gradient layer panels and fixed in place

using hot glue. The inner walls of the framing plastic were then coated with a layer of car wax, to prevent the frames from becoming permanently fixed to the epoxy. In the case of the top panel, which container holes in the middle of the panel for airflow, aluminum soda cans were stripped of their tops and bottoms and cut down one side, then rolled up to form smaller diameter circles to fit inside of the air holes. The final wiring and framing state of the thermopiles, prior to being poured in epoxy is shown in Figure 3.

Per the manufacturer's directions, the epoxy was poured and left for at least 24 hours to dry and harden – in some cases the epoxy took longer than the prescribed 24 hours to fully harden. Once the epoxy was hardened, the polycarbonate frames were removed from the panels. The finished gradient layers were then laid into place within the calorimeter. The base plate was simply laid flat in the bottom of the aluminum shell. The side walls were laid vertically on top of the base plate, with two small, clear Command Strips holding the tops of the walls to the aluminum frame. The long side walls were placed directly across from each other, and the short walls were placed in between them. A layer of 0.8 cm (5/16 inch) thick rubber foam, self-stick weather seal insulation (Frost King) was used to fill the gaps between all gradient layer panels to reduce air leakage from the calorimeter and to create a snug fit between panels to help hold them in place. The weather seal was also put into place on the tops of each side wall, where the top panel would sit. The top panel was not fixed into place, but rather simply sat on top of the four walls and insulation and would be squeezed snuggly into position when the top of the aluminum shell was screwed into place above the top panel. Weather seal was also placed on the top of the side walls of the aluminum frame to allow cushion for wiring to be run in and out of the calorimeter when the top was closed. The interior of the calorimeter after the thermopile panels were epoxied and put into place can be seen in Figures 4 and 5. The final dimensions of the interior of the calorimeter, after the epoxy was poured onto the gradient layer panels, were 16.8cm wide by 16.8cm long by 11.1cm tall (6.625 inches by 6.625 inches by 4.375 inches).

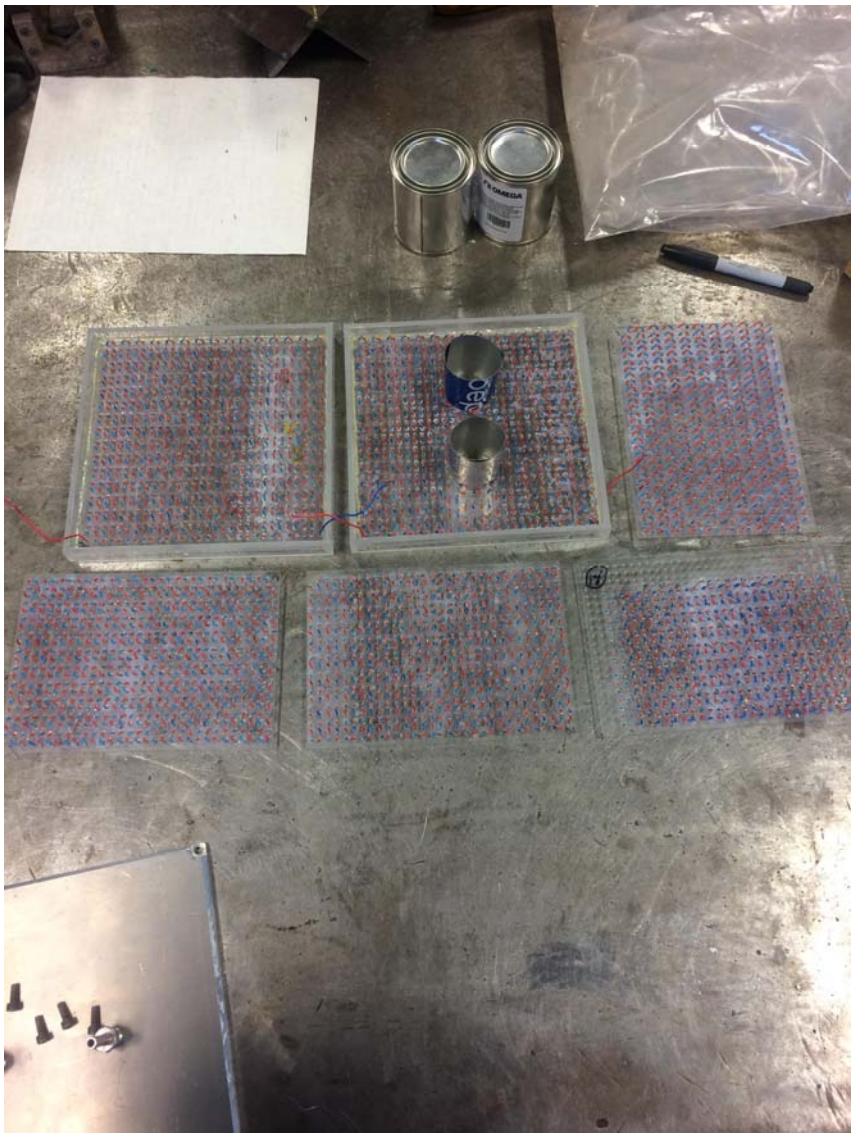

Figure 3. The six panels of the gradient layer with completed thermopile wiring. The bottom panel and top panel are framed and ready to be coated with epoxy.

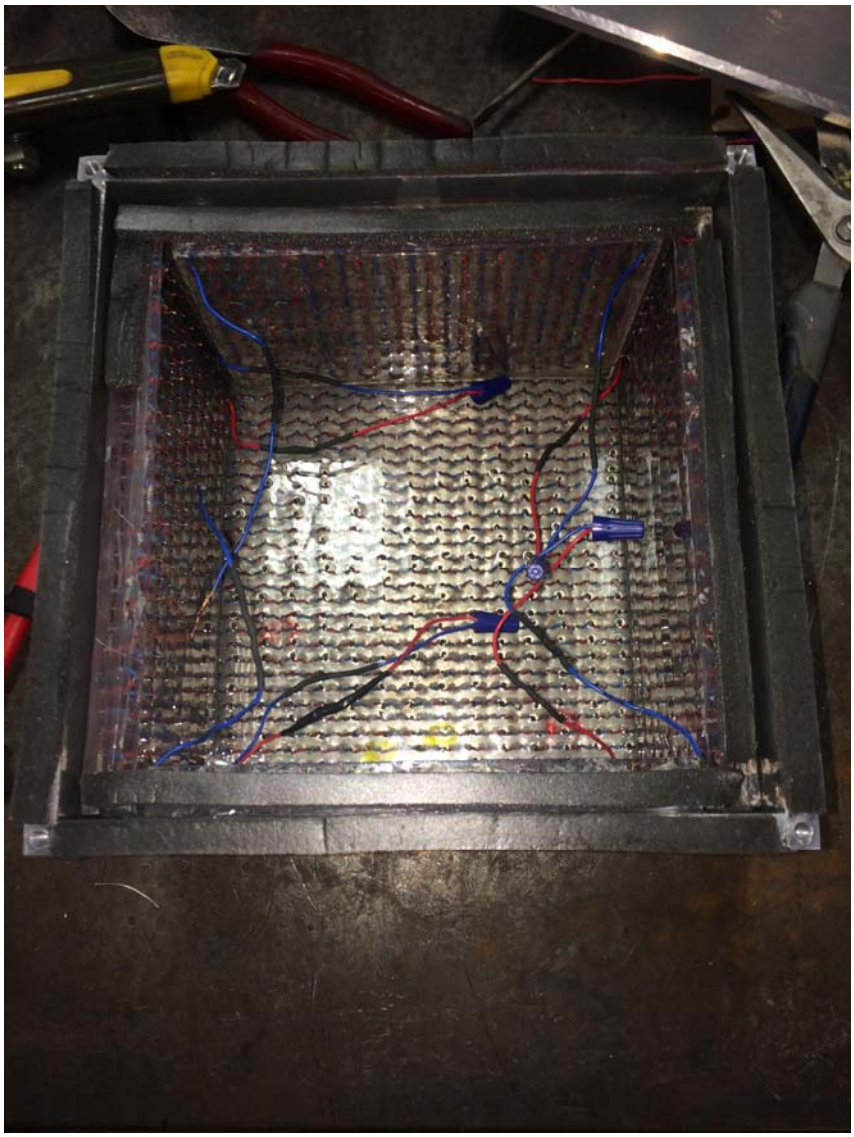

Figure 4. The interior of the calorimeter after all gradient layer panels were epoxied, put into place, and connected in series. During operation of the calorimeter, the wiring connection seen here in the middle of the chamber were fixed to the sides to avoid direct contact with the heat source inside the chamber.

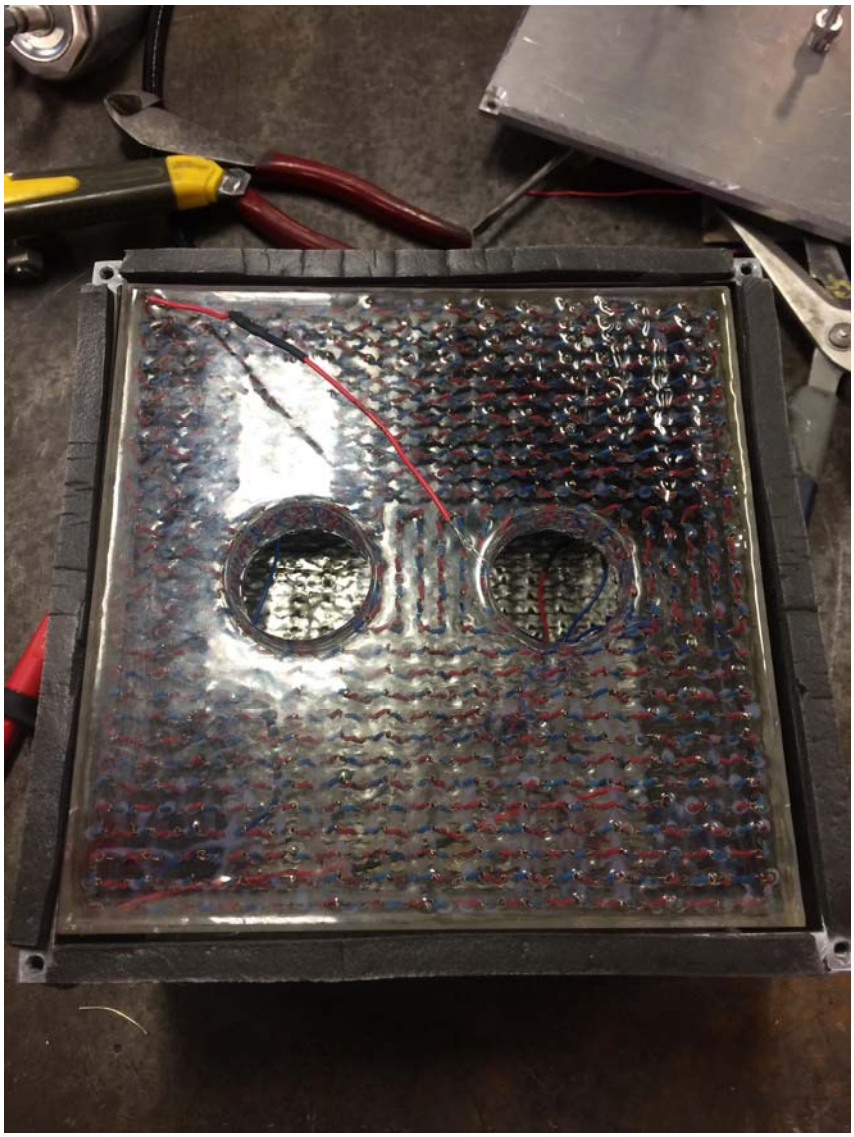

Figure 5. The inside of the calorimeter with the top panel in place.

Once all of the panels were put into place, the extra-long wires left exposed on each panel were connected to put all six thermopile panels into series with one another, as shown in Figure 6. Wires were not connected with wires of the same type – a copper wire from one panel would only be connected with a constantan wire from another panel. These wiring connections were held in place using wire nuts. Two of the long wires – one from the top of a side wall and one from the top panel – were left unconnected from the other panels. One of these wires was a constantan wire and the other copper. The wires were laid over the weather seal on the tops of the aluminum framing to exit the calorimeter when the top was screwed

into place. These two wires were connected to a Type-T thermocouple connector on the outside of the calorimeter. The thermocouple connector was then plugged into an Omega HH806AU Multilogger Thermometer, which was used to record data from the calorimeter.

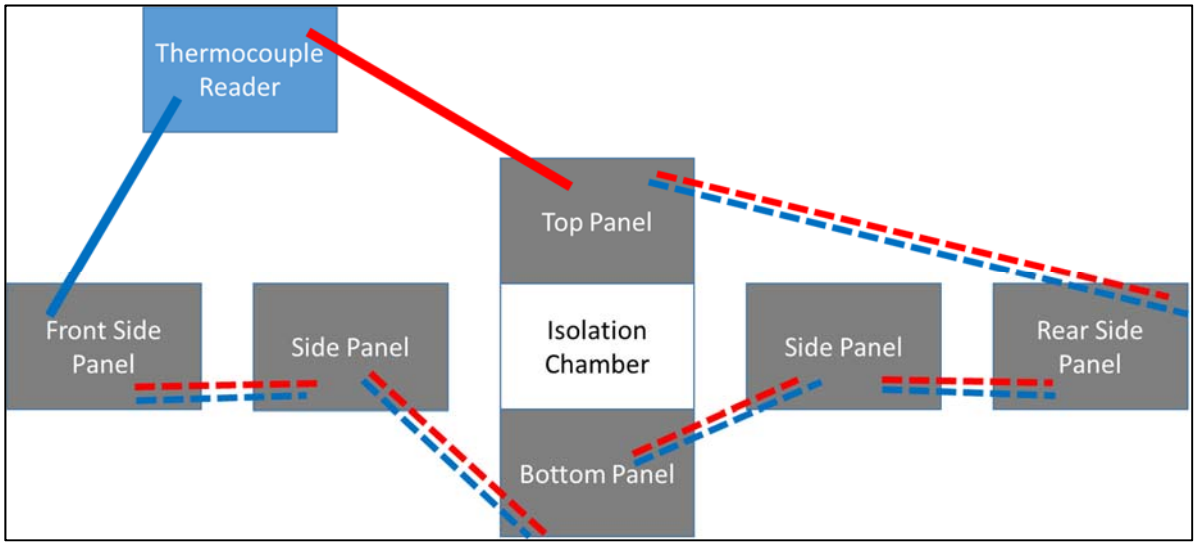

Figure 6. Wiring diagram of how the thermopiles of the calorimeter were connected in series to form a complete circuit, which then connected to a thermocouple reader. Solid wires indicate that a wire connection at that connection must be blue (copper) or constantan (red) wire to acquire proper readings. Dotted double lines indicate that connection wires in that location could be either copper or constantan, without affecting the voltage flow through the device.

Calibration Procedure

The gradient calorimeter constructed in this project was calibrated by exposing the interior of the calorimeter to a range of known heat production rates, letting the calorimeter reach an equilibrium where the amount of heat produced inside is the same as the heat leaving the outer shell, recording the readout of the thermocouple reader at equilibrium (when the output voltage stops climbing), and plotting the relationship between heat supplied and temperature (voltage) reading. The equation produced from this relationship could then be used to determine how many watts of heat were being produced within the calorimeter from the temperature reading recorded by the thermocouple reader.

To perform this calibration, the calorimeter was first placed in an environmental control chamber (Parameter Generation and Control, Inc., model

number 9295-22M4-9100000) and kept at a temperature and relative humidity of 27.0 ± 0.2 ^{0}C and 65 ± 3 % RH, respectively. A one foot long coil of nickel-chromium resistance heating wire (Omega Engineering, model number NI80-015-50), with a resistance of six ohms, was then suspended in the middle of the calorimeter chamber from two alligator cables (Figure 7), which were run into the calorimeter through the insulation on the tops of the walls. Care was taken to make sure the resistance wire was not making contact with any of the gradient layer panels. The alligator cables were plugged into an ExTech 80W Switching DC power supply, which sat outside of the calorimeter. The thermopile wires that extended out of the calorimeter and connected to a thermocouple connector, were then plugged into the Omega Engineering HH806AU Multilogger Thermometer, which produced a temperature readout corresponding to the voltage measurement registered from the thermopile. The entire calibration setup can be seen in Figure 8.

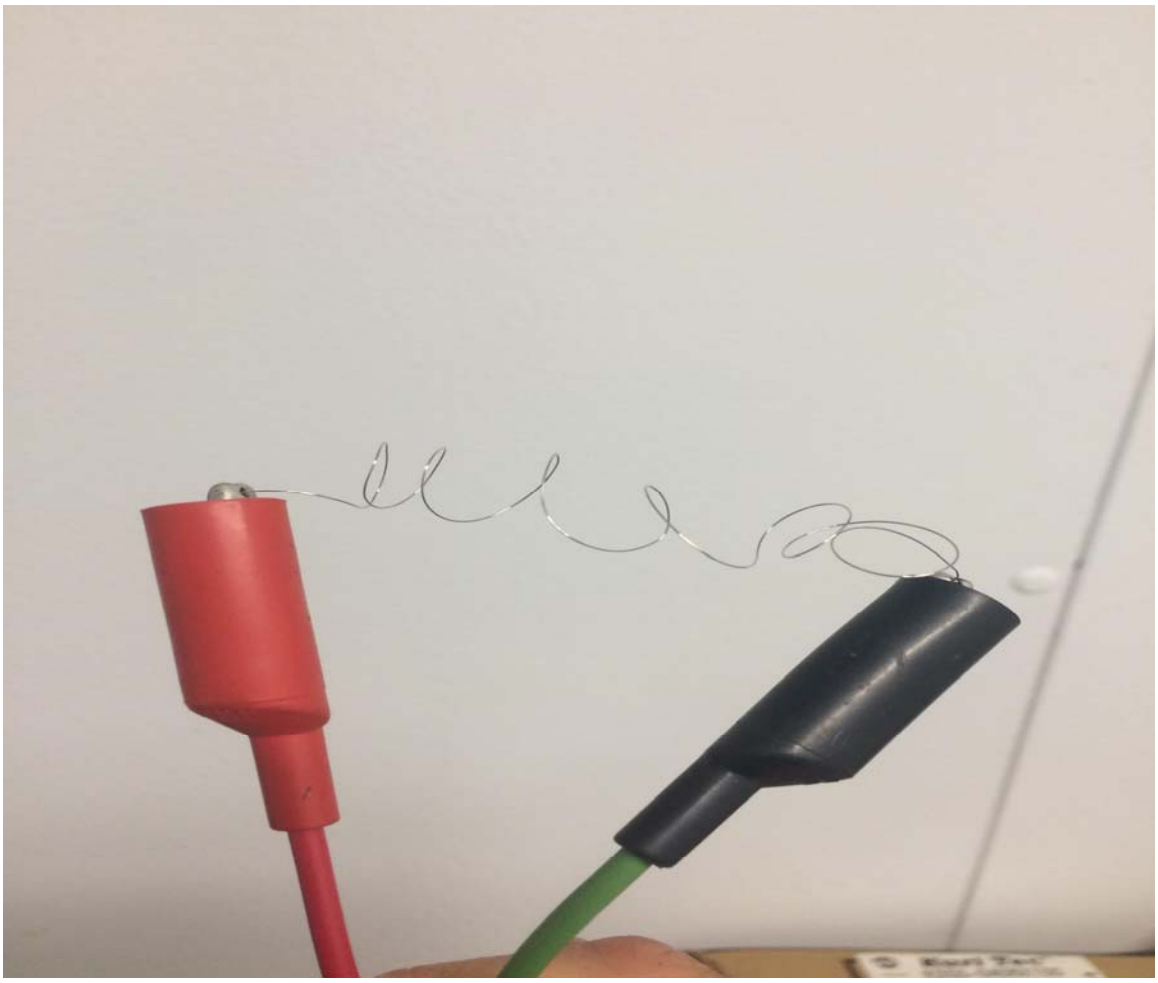

Figure 7. The heating resistance wire and alligator clips used to create a known heat production rate inside of the holding chamber of the calorimeter during calibration.

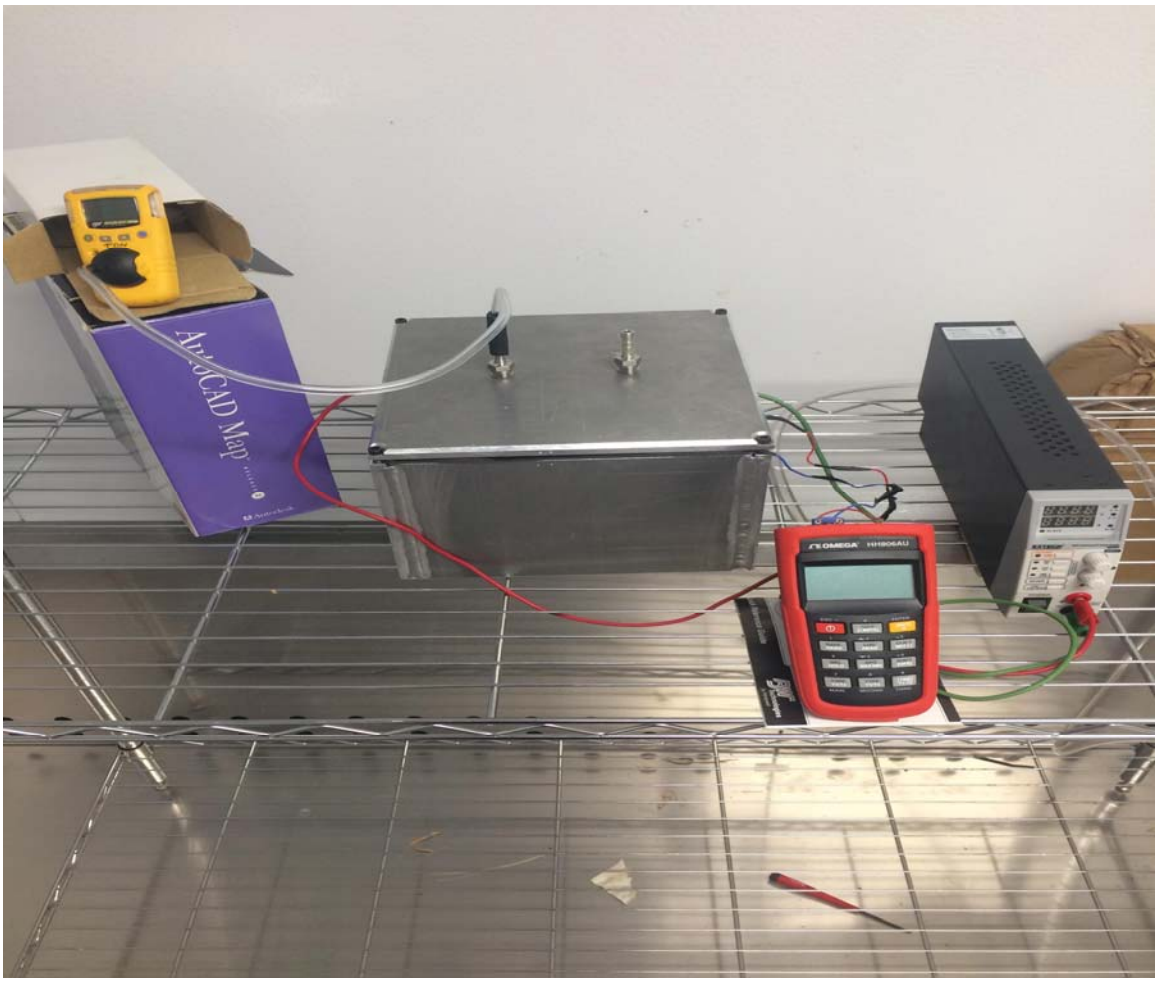

Figure 8. The calibration set up including the DC power supply (right) connected by alligator clips to the resistance heating wire (suspended inside the chamber), the calorimeter wires attached to the thermocouple connecter and plugged into the thermocouple reader (center), and the ammonia sensor (left) attached by plastic tubing to the outflow air tube in the top of the calorimeter.

The thermometer was designed to determine temperature from a single thermocouple, not a large thermopile, so the temperature readouts were not accurate. However, since the thermometer's temperature readouts corresponded with the voltage produced by the thermopile and the voltage produced by the thermopile corresponded to the heat produced within the calorimeter, the heat production rate inside and the thermometer temperature readout could be correlated mathematically. Therefore, once the assembly described above was prepared, known amounts of heat were passed into the calorimeter using the

resistance heating wire. The heat production rate produced by the wire was determined using the Power Law shown in Equation 3:

$$P = I * V \qquad (3)$$

where P is power, or in this case heat production rate, in watts; I is amperage in amps; and V is voltage in volts. The amps and volts flowing through the wire were known and controlled using the ExTech power supply. Since the wiring was not connected to any other electrical load, it could be assumed that all of the power that passed through the resistance wire was dissipated as heat. Therefore, the heat production rate produced within the calorimeter was known.

The wire was allowed to produce heat at a given rate within the calorimeter for a prolonged period of time, until the temperature readout on the thermometer reached equilibrium. Equilibrium was assumed once the temperature readout stabilized, or stopped changing. At this point, it was assumed that the amount of heat leaving the calorimeter was equal to the amount being added by the wire and both the heat production rate through the wire and the temperature readout from the thermometer for that point were recorded. This process was repeated for a range of heat production rates between 0 W and 2.5 W, and the results were plotted against each other. The resulting plot is shown in Figure 9.

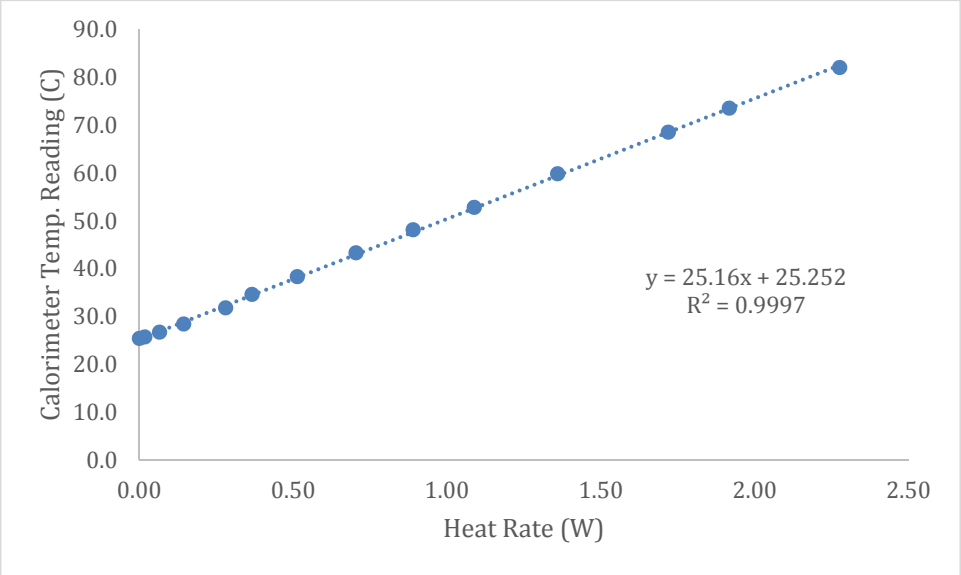

Figure 9. The calibration curve and equation between heat production rate and temperature readout from the calorimeter. This calibration was performed with one airflow port closed and the other attached to an ammonia sensor.

Rearranging the results from Figure 9 yielded Equation 4, which was used to determine the heat production rate being produced within the calorimeter during experimentation with BSFL.

$$X = 0.0397y - 1.0032 \qquad (4)$$

where x is the heat production rate within the calorimeter in watts and y is the temperature readout from the thermocouple reader, in degrees Celsius.

Using this equation, the heat production rate produced by organisms within the calorimeter could be determined from only the temperature readout of the thermometer. Once the calibration process was completed, the calibration curve was validated by applying a random amount of heat from the power supply and checking to make sure that the amount of heat predicted by the calibration curve matched the actual heat being applied.

The ExTech power supply had a reported voltage and amperage accuracy of ±1%. Therefore, from applying the accepted error to the power calculation, the resulting heat production measured by the calorimeter should been within 0.01 W of the actual heat production on the lower ends of the calibration curve and within 0.045 W on the higher end of the calibration curve. When the machine was

calibrated, it was unknown how much heat the BSFL would produce, which is why the calibration curve was extended out to around 2.5 W of heat. As seen in the results section, the largest amount of heat that was produced by single BSFL group was 0.407 ± 0.01, therefore, all of the BSFL data measurements fell within the lower ranges of the calibration curve. Equation 5 shows the calibration equation with error included.

$$X = (0.0397y - 1.0032) \pm 0.01W \qquad (5)$$

It should be noted that the above data represents only one calibration scenario. In this calibration, one of the airflow ports in the top of the calorimeter was connected to additional tubing, and a passive ammonia sensor (the ammonia sensor did not actively pump any air), while the second air port was plugged to prevent airflow. Four other calibrations were conducted using the same process, but with different scenarios in regards to the airflow ports. In the first scenario, the calibration procedure was followed, with the first air flow port left open while the other was attached to the passive ammonia sensor. In the second, both air flow ports were completely sealed to prevent airflow into and out of the isolation chamber. In the third, fresh air was forced through the chamber at a rate of 18.5 air exchanges per hour (approximately 970 mL/min), using a rotameter (Dwyer Instrument, 0.7—5.0 $L*min^{-1}$). In the fourth and final scenario, fresh air was forced through the chamber with a different rotameter (Omega Engineering, 0.1—100 $mL*min^{-1}$) at an air exchange rate of 1.65 air exchanges per hour (approximately 85 mL/min). The calibration curves for these four scenarios are included as Appendix A.

The appropriate airflow scenario for a given test and calibration depends on how much oxygen is required by the study organism to survive during testing. If the organism consumes little oxygen and can survive the duration of the test without needing fresh air, then calibration with plugged ports may be most appropriate, as was the case in this experiment. If the organism needs fresh air inputs into the chamber to survive the testing period, then the calorimeter should be calibrated in the same way as described above, but with fresh air being pumped through the

chamber at a known rate, so that the calibration may account for the heat lost as air moves through the chamber.

It should also be noted that this device should be re-calibrated any time it is moved into a room with a different ambient temperature. The device's heat production rate measurements are based off of conductive heat flow through the walls of the device, as described by Fourier's Law, shown in Equation 6:

$$Q' = -kA(T_h - T_c) \qquad (6)$$

Therefore, the outer temperature will play a factor in the rate at which heat moves through the gradient layer and the measurement obtained by the thermocouple reader. So if the exterior temperature of the calorimeter is changed, the readings will not be accurate without a new calibration. As long as the external temperature of the calorimeter is kept constant, however, tests can continue to be conducted without repeated calibrations.

Results of Calorimeter Build

After calibration, this calorimeter was used to successfully measure the heat production of black soldier fly larvae (BSFL), *Hermetia illucens.* During testing, just like the calibration, the calorimeter was housed inside a controlled environment chamber, where the ambient conditions were maintained at a steady 27°C and 65% RH. The BSFL measurements were recorded using the original calibration scenario discussed above and described by Figure 9.

The calorimeter was successful in recording heat production rates as low as 0.02 W during calibration. The device recorded heat production rates as low as 0.01 W during BSFL testing, however, readings this low were not used, since they fell outside of the calibration curve. Likewise, the highest heat production rates successfully measured during calibration and BSFL testing were 2.45 W and 0.41 W, respectively. Moreover, the BSFL measurement results behaved as expected, showing a steady increase in temperature within the calorimeter over a period of time, before reaching an equilibrium point, at which the heat production rate within

the calorimeter leveled off. An example of data from one BSFL trial run is shown in Figure 10 in the Results and Conclusions section.

Objective 2 – BSFL Rearing and Data Measurements

Rearing Containers and Environmental Control for BSFL Upkeep

The BSFL were kept an environmental chamber (Parameter Generation and Control, Inc., model number 9295-22M4-9100000) and kept at a temperature and relative humidity of 27.0 ± 0.2 ^{0}C and 65 ± 3 % RH, respectively. Artificial lighting was available in the controlled environment chambers, however, lighting is not mentioned in the literature as having an important impact on the growth and development during the larval stages, therefore lighting schedules were not observed or monitored.

It has been suggested that BSFL will grow and develop well in population densities of ~2.5 larvae per cm^2 (Sheppard et al., 2002), although at least one study has suggested densities as high as five larvae per cm^2 to be acceptable (Denier et al., 2009). A population density of ~2.5 larvae per cm^2 was maintained during data collection for this study, while populations of 5,000 larvae were housed in tall plastic containers with 35.5 cm by 30 cm bottoms (14-in by 11-in), and about 30.5 cm (1ft) tall walls when not being tested in the calorimeter.

Feeding

Chicken feed has been identified as a high quality feed source for BSFL, and optimal feeding rates for BSFL have been identified in previous studies (Sheppard et al., 2002; Denier et al., 2009). Therefore, a 15% protein layer hen feed was fed to the BSFL in this experiment at a rate of 100 mg per larva per day, which was concluded to be an ideal feeding rate from Denier et al. (2009).

Since BSFL take in water from their food, moisture was added to the feed. Sheppard et al. (2002) suggests using chicken feed with a 60-70% moisture content. For this experiment, a 60% moisture content was used. Therefore, for every 100 mg of feed, 60% was water and 40% was dry feed, by weight. Consequently, 500 g of

fresh feed was given to the entire population of 5,000 larvae each day, at a 3:2 water to dry feed ratio. For those larvae which were randomly selected for testing on a given day, the amount of food appropriate for that sized aggregate was removed from the rest of the food and stored until the group was ready to be placed into the calorimeter. When the group was ready for testing, the appropriately portioned feed was placed into the testing tray with the larvae and went into the calorimeter with the larvae during testing to ensure the larvae would be under normal behavior. For example, if a group of 100 larvae was being tested, then 10 g of fresh feed removed from the larger feed pile and was placed in the testing tray with the 100 group.

Studies on other species of fly larvae have demonstrated that feeding old anaerobic organic waste, or even several day old aerobic waste, can result in significantly less larval growth and can even be lethal to the larvae (Beards and Sands, 1973). Therefore, fresh feed was added daily in this study and the holding container was cleaned of previous food waste and BSFL frass twice per week. These waste samples were dated and kept in freezer storage for further ammonia and nitrogen content testing after the calorimeter measurements were finished.

Extraction for Calorimetry and Experimental Timeframe

Though not conducted using BSFL larvae specifically, experiments have shown that aggregations of fly larvae have the potential to create significant increases in temperature in the immediately surrounding air (Heaton et al., 2014). Consequently, this project measured the heat production of several different aggregate sizes: 100, 300, and 500 larvae. To maintain ideal population densities of 2.5 larvae per cm^2, holding trays with floor dimensions of 6.35cm-by-6.35cm, 14.60cm-by-8.25cm, and 14.60cm-by-12.70cm (2.5”x2.5”, 5.75”x3.25”, and 5.75”x5”) were used to house the 100, 300, and 500 larval aggregates, respectively, during testing.

Measurements began on the first day with a randomly selected aggregate size (100, 300, or 500). Each aggregate was counted out by hand, placed into the appropriate holding tray and placed in the calorimeter on top of small rubber stoppers to prevent direct contact with the thermopiles. The larvae were placed into

the calorimeter with fresh food and measured for 2.5 hours. Since the larvae and food were kept within the environmental control chamber prior to being introduced to the calorimeter, it was assumed that the temperature of the larvae, food, and holding tray were all 27 ^{0}C when measurements were initiated, as was the calorimeter itself. Heat production data collected during the first half hour was not used in data analysis, to prevent any heat data fluctuations that could be associated with the experimenter entering and leaving the environmental chamber while prepping the larvae. However, ammonia data was collected for the entire time period using the ammonia sensor attached to the outflow air hole in the top of the calorimeter. The other port of the calorimeter was sealed to reduce variance from extra air flow into and out of the calorimeter. (The larvae survived in the calorimeter without the need for additional fresh air.)

Heat production data was collected every minute for the remaining two hours using the Omega thermocouple reader. After two hours, data recording was stopped and the larvae were moved back into the larger rearing container. The same procedure was followed with the other aggregate sizes. Larvae were always selected at random from the overall population for each trial. Measuring each aggregate size multiple times throughout lifetime of the larvae not only produced replicate data from the experiment, but also allowed for comparison differences in heat production at different larval ages. After data was collected, the heat production rate was calculated using the calibration curve, and ammonia data was collected from the ammonia sensor. Since the feed put into the calorimeter for each measurement was fresh, it was assumed that all of the heat measured produced by the BSFL alone, and no microbial or anaerobic digestion contributed to heat production.

Additionally, every day that heat production rate measurements were taken, a random sample of 100 larvae were counted out and weighed. The weights of the larvae that day were recorded and used determine how the larvae's heat production changed in relation to the larvae's weight.

Data Collection Procedure

After the calibration was completed, the heat production rates produced by the BSFL groups were measured by placing a group within the calorimeter and firmly closing and tightening the top with the group inside. Once the BSFL were inside, the heat production rate produced was determined from the thermometer readings and the calibration equation (Equation 4). The thermocouple reader used in this study contained a data logging function. Therefore, heat production rate data could be attained for the entire period. Heat production rate was then plotted against time to determine when the heat production rate in the calorimeter stabilized. Stabilization was determined by comparing the slopes of the last 30 minutes of data collection to the slope of the entire curve, as well as to a slope of zero. If the slope of the last 30 minutes of the curve was significantly lower than the slope of the overall curve, and not significantly different than zero, then the heat production rate was said to have stabilized. (A visualization of the difference in slopes is provided in Figure 10.) The average heat production rate of the last 30 minutes was then used as the total heat production rate given off by that group of BSFL.

The airflow port was used to measure ammonia production of the BSFL during feeding and normal activity. In this study, BSFL were able to survive in the calorimeter during the measurement periods without the need for fresh air to be pushed through the system. Therefore, like in the calibration described above, one air port was sealed and the other was connected to additional tubing and a BW Technologies GasAlert Extreme Single Gas Detector ammonia sensor. The ammonia sensor ran throughout the duration of each sampling period when the BSFL groups were inside of the calorimeter and measured changes in the gaseous ammonia concentration.

Additional materials, such as the ammonia sensor and tubing, were not allowed to make direct contact with the main body of the calorimeter's outer shell. Such contact could potentially increase conduction rates and change the temperature of the aluminum in the area immediately around where the contact occurs. A temperature shift like this, even if small, is likely to be detected by the

thermopiles inside, and may alter the accuracy of the device. Likewise, the BSFL and testing trays were also not allowed to make direct contact with the gradient layer panels while inside of the calorimeter. Direct contact would cause an uneven and extreme variation of heat dissipation within the calorimeter and could potentially reduce the accuracy of the measurements. Therefore, the testing tray was placed onto minimally-conductive rubber stoppers while inside the calorimeter.

After data collection was completed, SAS 9.4 statistical software was used to run linear regressions to determine relationships between heat production, age, weights, and group size of the larvae, using PROC GLIMMIX procedures. PROC CORR functions were also used to check correlations between age and heat production variables. To perform comparisons between different ages and weight, the SAS program categorized ages and weights into distinct groups before the analysis. This allowed us to compare the heat production of larvae which were ”young” and “small” against larvae which were “old” and “large.” It was acknowledged that age and weight of the larvae are collinear, however, both were still measured and correlated with heat production, because we were not confident which variable would be the most practical and most accurate for estimating the heat production from BSFL within a facility.

After Pupation

At the conclusion of each round of the study (when the larvae reached pupation), they were removed from the container and frozen. Some samples of larvae were randomly selected and kept at freezing temperatures in case they needed later for further analysis. Afterwards, the majority of the population was disposed.

Results

Heat Production

Heat production, larvae age (in days), and larvae weight (per 100 larvae) were recorded throughout data collection. Linear regression analysis conducted using SAS 9.4 statistical software determined that there was a significant relationship between the age of the black soldier fly larvae and the amount of heat produced. It also determined that there was a significant difference between the heat production between the heaviest and lightest BSFL groups.

Total Heat Production

As expected, the largest groups of larvae produced the greatest amounts of total heat during this study. The maximum heat produced by each trial was determined from the average heat production rate during the last 30 minutes of the trial, after the heat production rate had leveled off, as demonstrated in Figure 10. Figure 11 shows that, as expected, the amount of total heat recorded by the calorimeter increases with the total amount of larvae inside. The greatest heat production observed during the study was 0.407 W, which was produced by a group of 500 larvae. The average total heat production throughout the lifespan of larvae in groups of 500 was 0.247 W, which was significantly more than the average total heat production of groups of 300 and 100 larvae, throughout their lifespan: 0.200 W 0.107W, respectively.

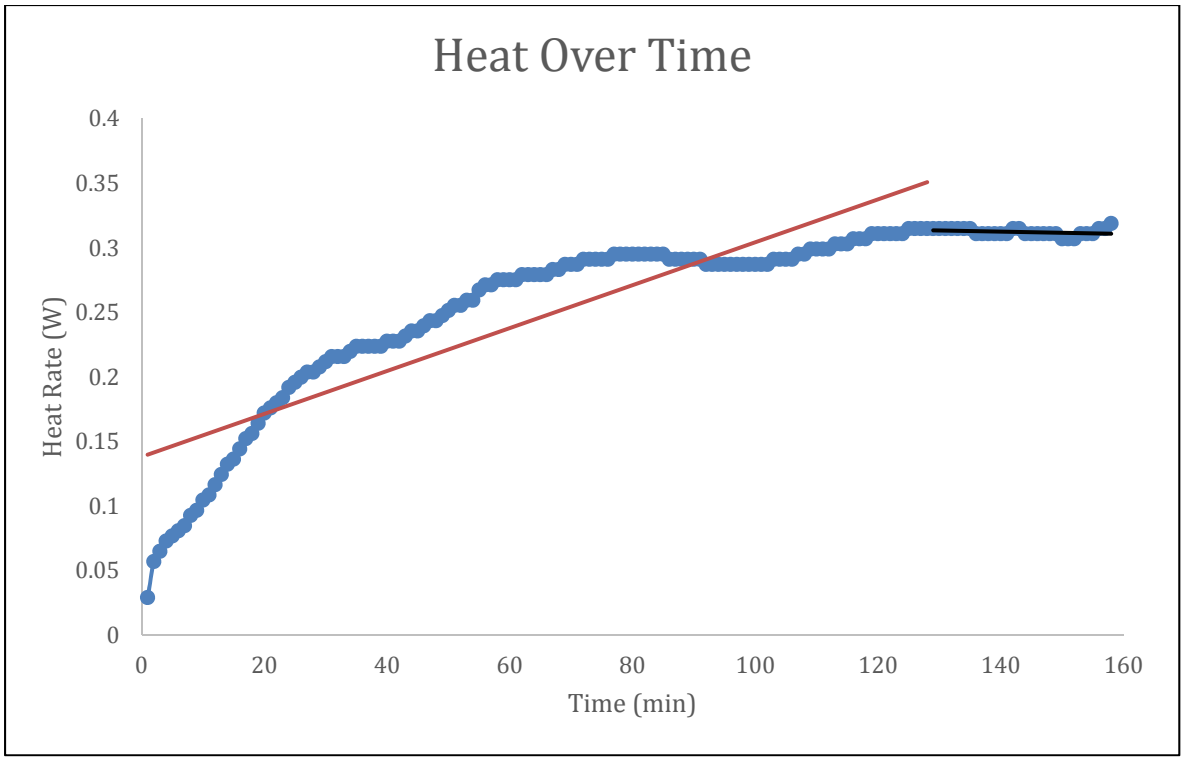

Figure 10. The heat production rate of a group of 500 BSFL over time. As demonstrated in the chart, the heat production rate eventually reaches a plateau. The blue curve represents the raw data points collected during this measurement. The orange and black lines represent the slopes first 128 and last 30 minutes of the data, respectively. Because of the significant difference in the slopes, it was assumed that the average heat production rate of the last 30 minutes of the data was the maximum heat production rate for this trial.

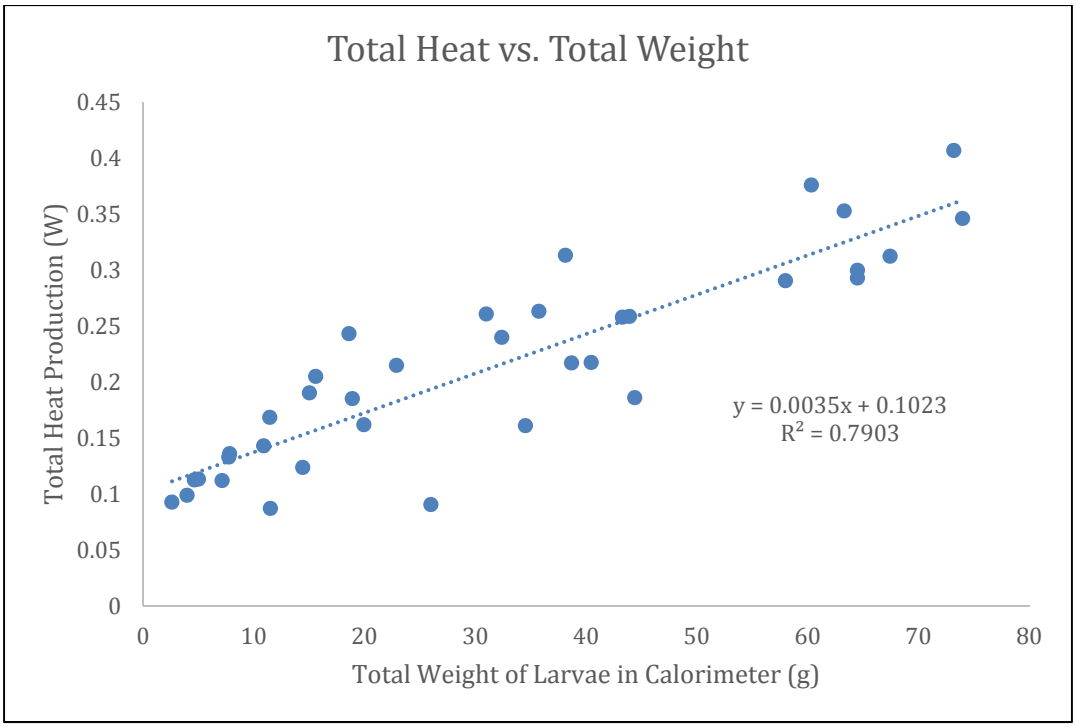

Figure 11. Total heat production plotted against the total weight of the larvae in the calorimeter.

Age vs. Heat Production

The age of the larvae was significantly correlated to total heat produced, heat produced per individual larva, and heat produced per gram of larva (Table 1).

Table 1. P values yielded by SAS Glimmix function for the relationship between age and heat productions

	Total Heat (W)	Heat Per Larva (W)	Heat Per Gram of Larva (W)
Larvae Age (Days)	0.0013	0.0208	<0.0001

As the age of the larvae increased, the total heat produced and the heat produced per individual larvae also increased (Figures 12, 13, 14, and 15). However, as the age of the larvae increased, the amount of heat produced per gram of larvae decreased (Figure 16).

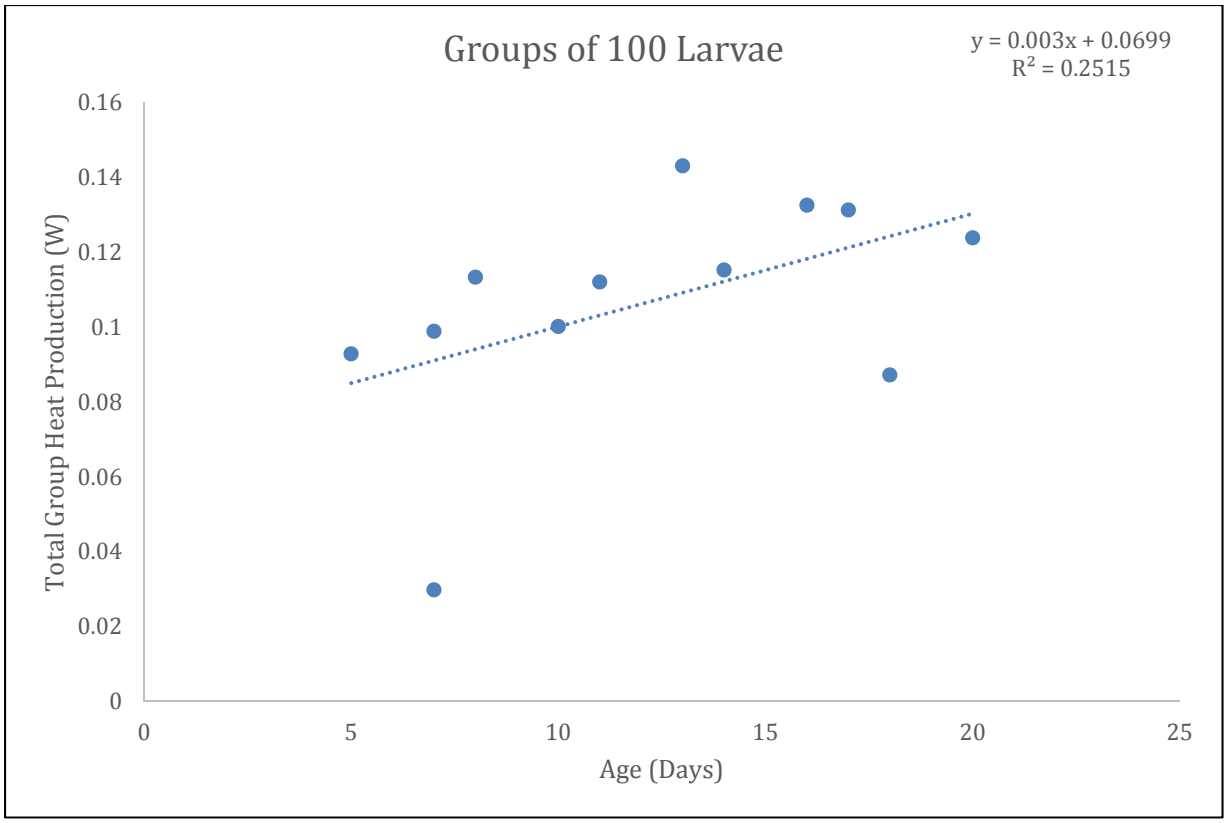

Figure 12. The heat production of the group increases with the age of BSFL in groups of 100 larvae

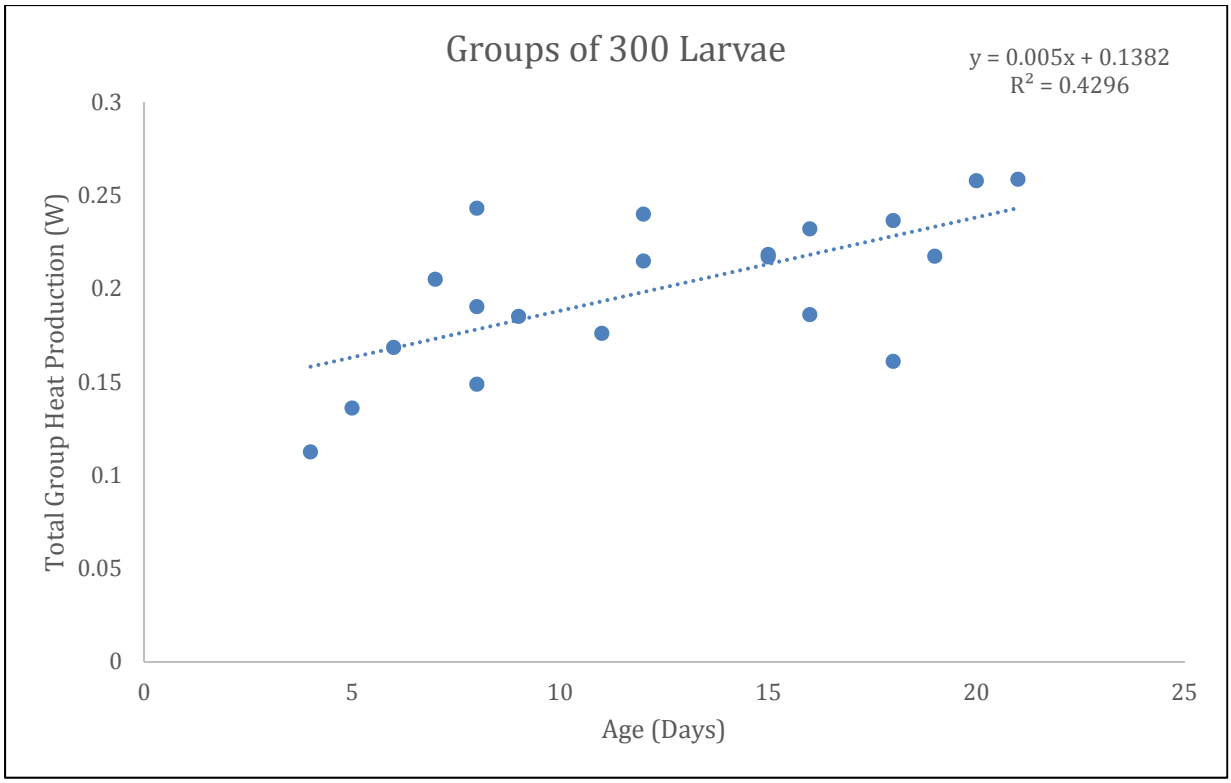

Figure 13. The heat production of the group increases with the age of BSFL in groups of 300 larvae

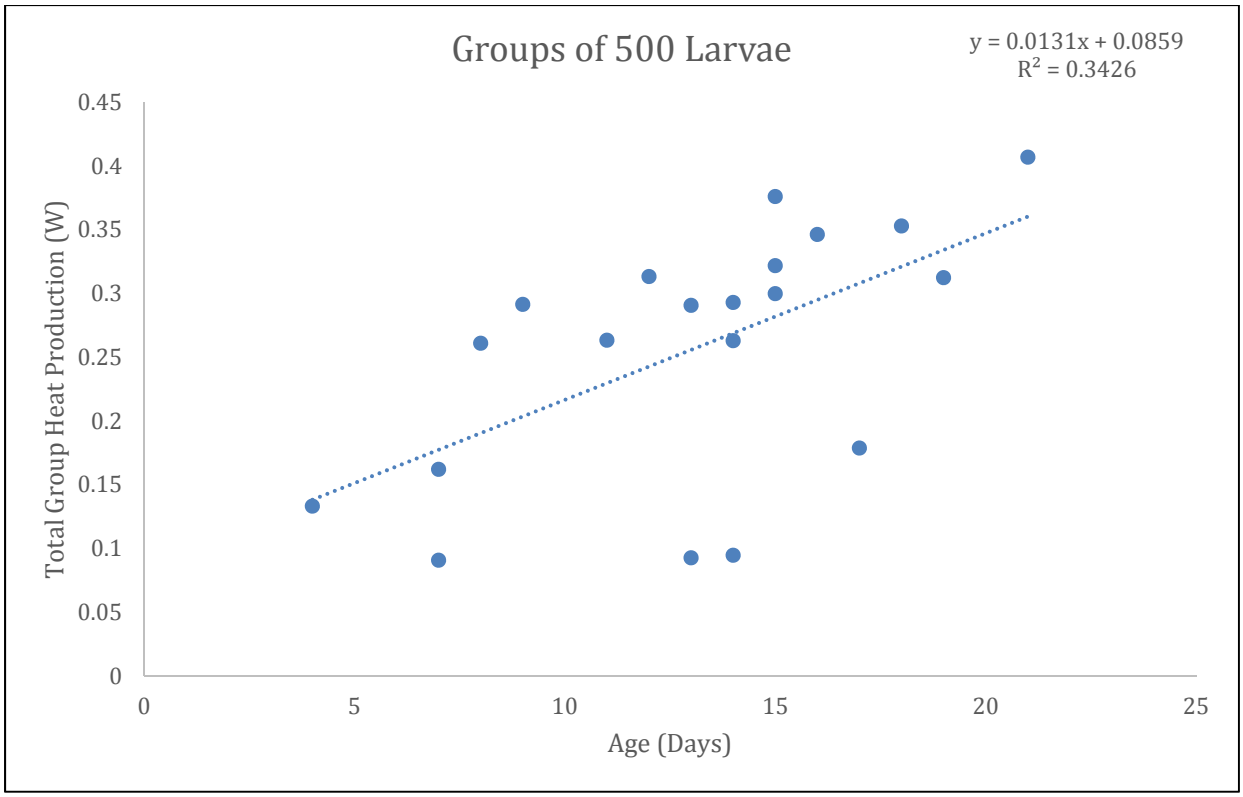

Figure 14. The heat production of the group increases with the age of BSFL in groups of 500 larvae

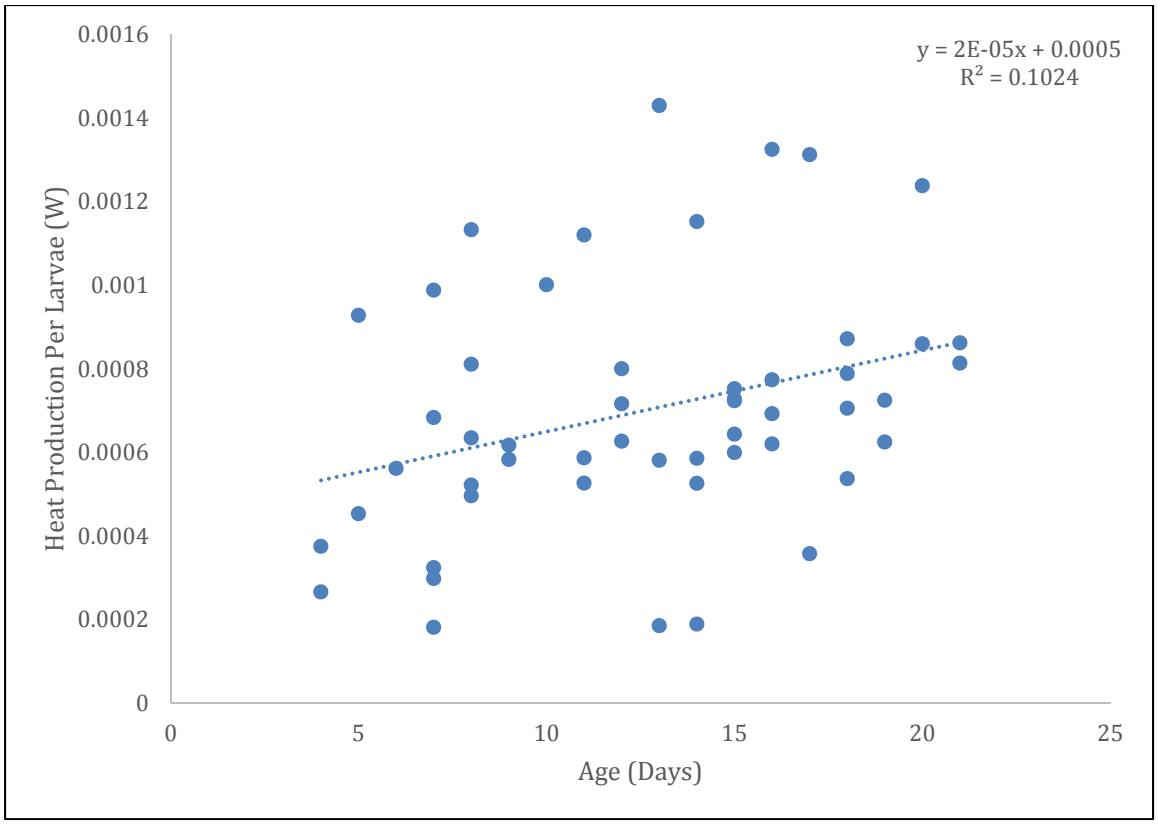

Figure 15. The heat production per individual larva increases with age

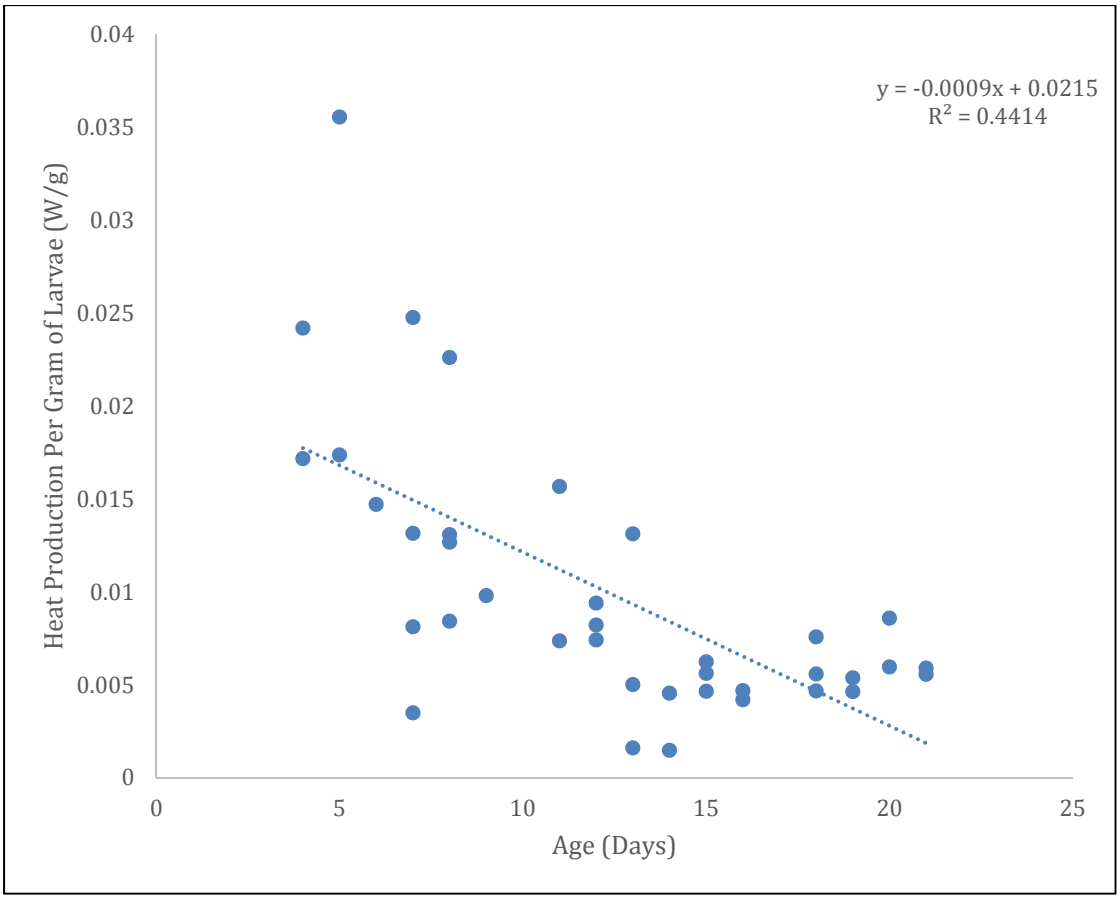

Figure 16. The heat production per gram of larvae decreases with age.

Weight vs. Heat Production

For the analysis of larval weight, the larvae were separated into five weight categories: 0-3, 3-6, 6-9, 9-12, and 12-15 grams per 100 larvae. Table 2 provides a visualization of the different weight categories, and the average heat production for heat weight class on an individual and per gram basis. The statistical analysis found that the heat produced was also significantly correlated with the weight of the larvae (Table 3).

Table 2. Average heat production per gram and per larva for each weight category.

Weight Category (g)	Number of Replicates	Mean (W/g)	Standard Deviation	Mean (W/Larvae)	Standard Deviation
0-3	4	0.02356	0.00863	0.00051	0.00029
3-6	7	0.01421	0.00750	0.00064	0.00034
6-9	7	0.01027	0.00300	0.00071	0.00021
9-12	6	0.00656	0.00388	0.00073	0.00042
12-15	14	0.00521	0.00153	0.00071	0.00022

Table 3. P values from SAS for the relationship between weight and heat productions

	Total Heat (W)	Heat Per Larva (W)	Heat Per Gram of Larva (W)
Weight (g)	0.0084	0.0018	<0.0001

SAS correlation and GLIMMIX analysis determined which weight categories yielded significant differences in heat production:

- Only the lightest weight category (0-3 g/100 larvae) and the heaviest weight category (12-15 g/100 larvae) had significant differences on their effect on the total heat production. In other words, although the general trend was that overall heat production increased as the weight of the larvae increased, most weight categories did not yield significantly different amounts of total heat.
- Similarly to overall heat production, only the lightest and heaviest weight categories resulted in a significant difference in the amount of heat produced per individual larva. In other words, larvae in the 12-15 and 0-3 grams/100 larvae categories had significantly different amount of heat production per individual larva – the heaviest category producing the most heat – while larvae in the categories in between did not yield significantly different amounts of heat per individual.
- In terms of heat production per gram of larvae, three weight categories had significant differences. Larvae weighing between 0-3, 6-9, and 9-15 grams per 100 larvae all yielded significantly different amount of heat per gram of larvae, with the heaviest category producing the least heat per gram and the lightest category producing the most.

In general, as the weight of the larvae increased, the amount of total heat and heat per individual larva increased (Figures 17, 18, 19, and 20). However, like with age, as the weight of the larvae increased, the amount of heat produced per gram of larvae decreased (Figure 21).

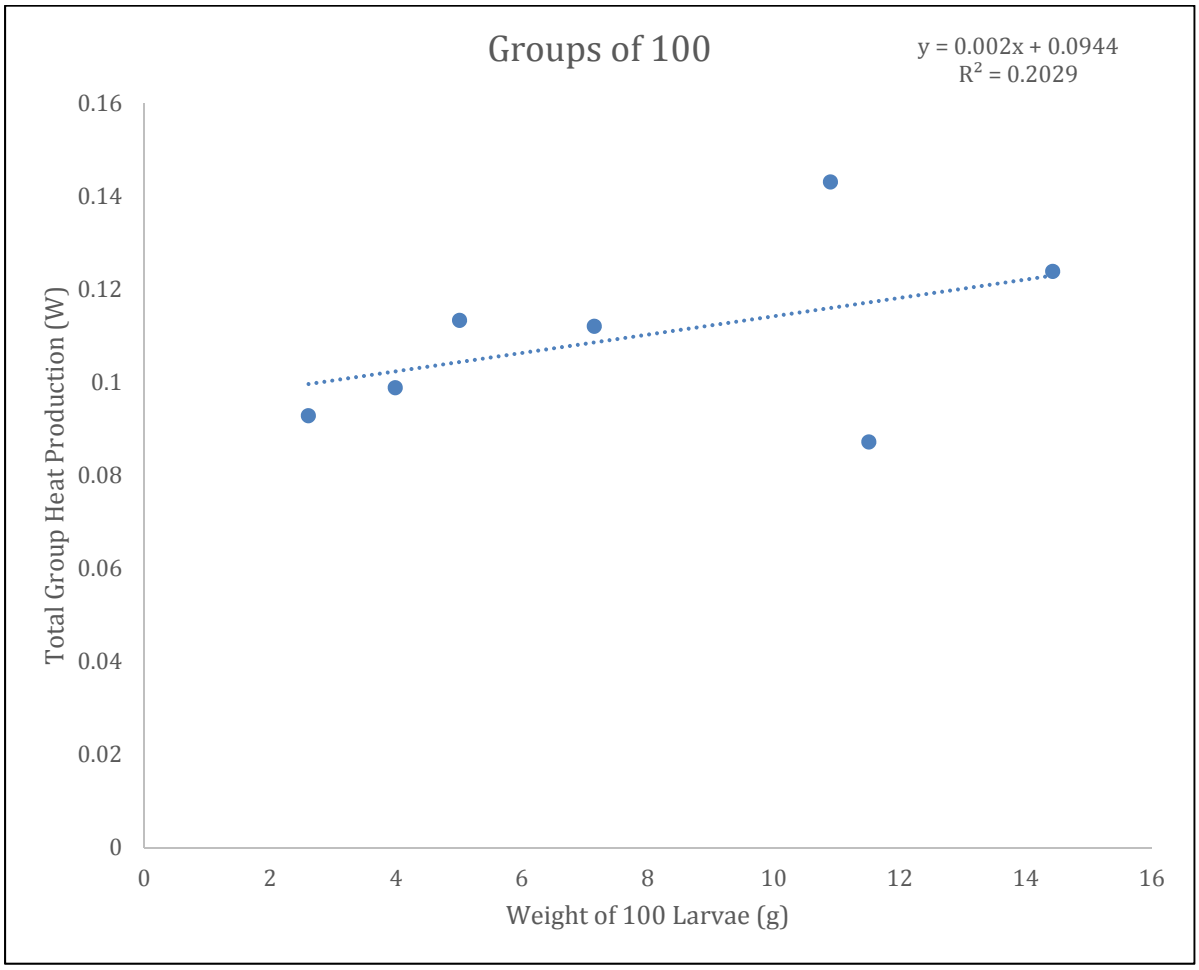

Figure 17. The heat production of the group increases with the weight of BSFL in groups of 100

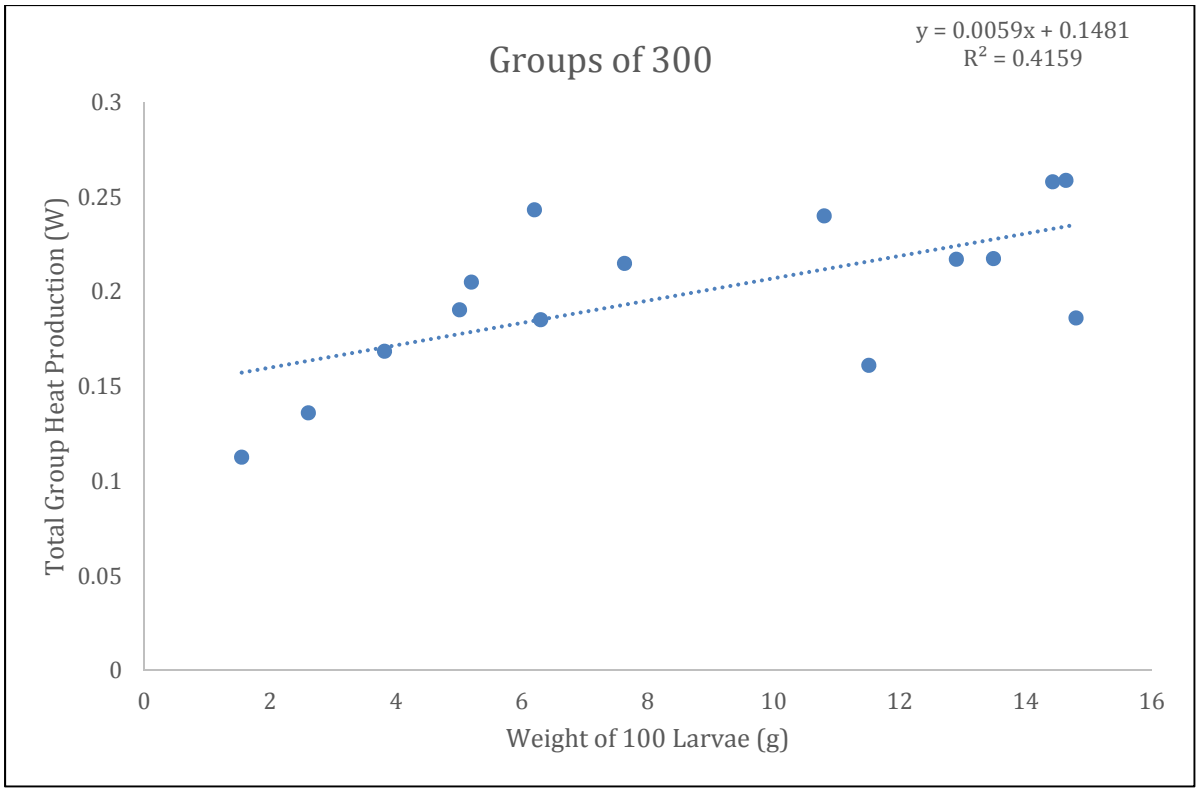

Figure 18. The heat production of the group increases with the weight of BSFL in groups of 300

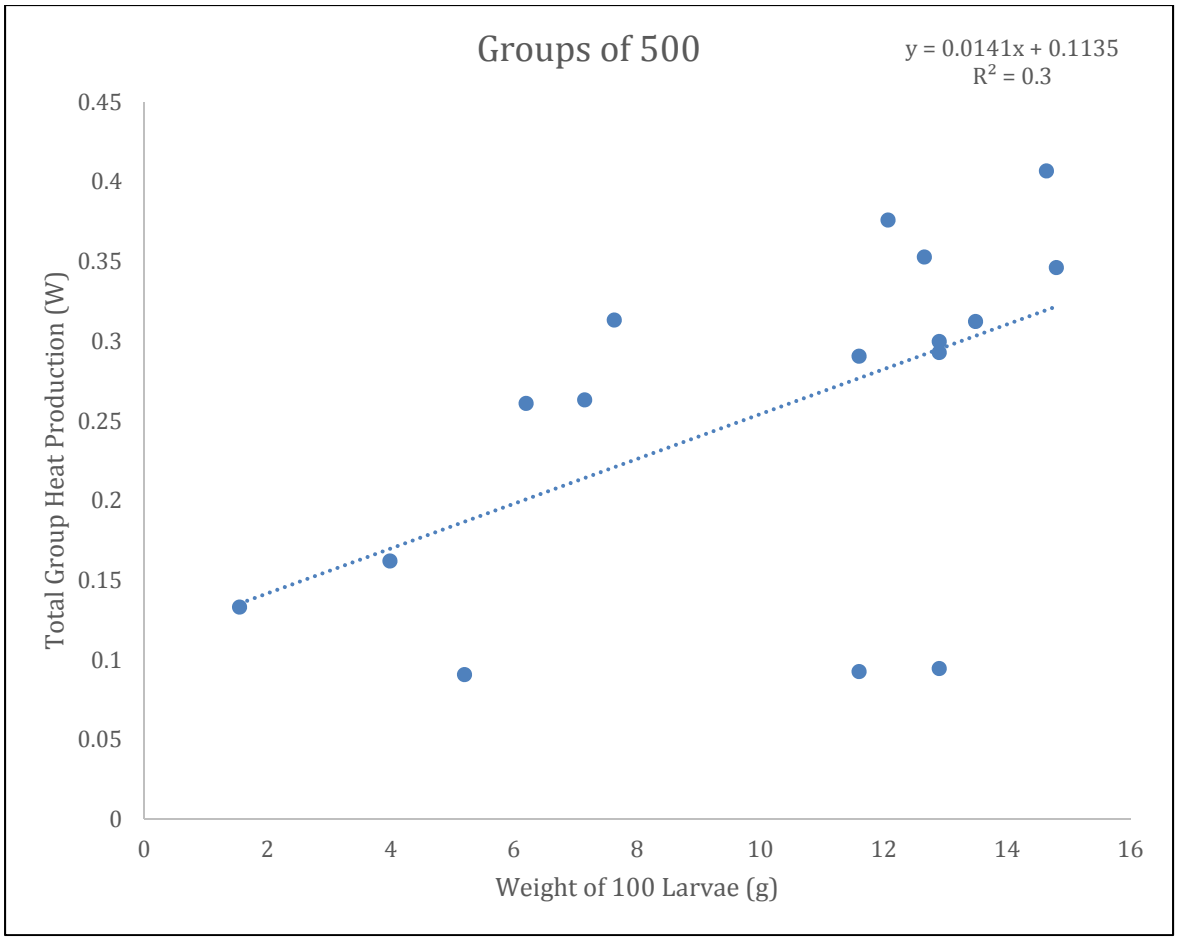

Figure 19. The heat production of the group increases with the weight of BSFL in groups of 500

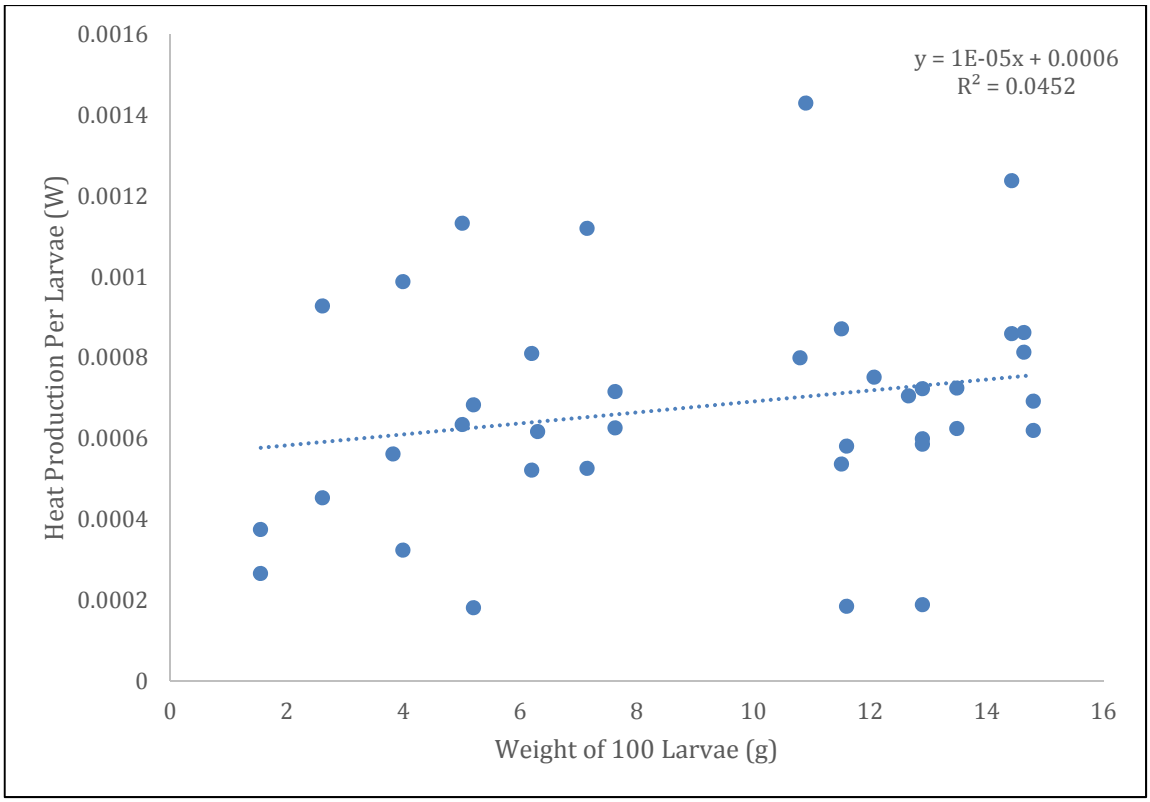

Figure 20. The amount of heat per individual larva increases with the weight of the larvae

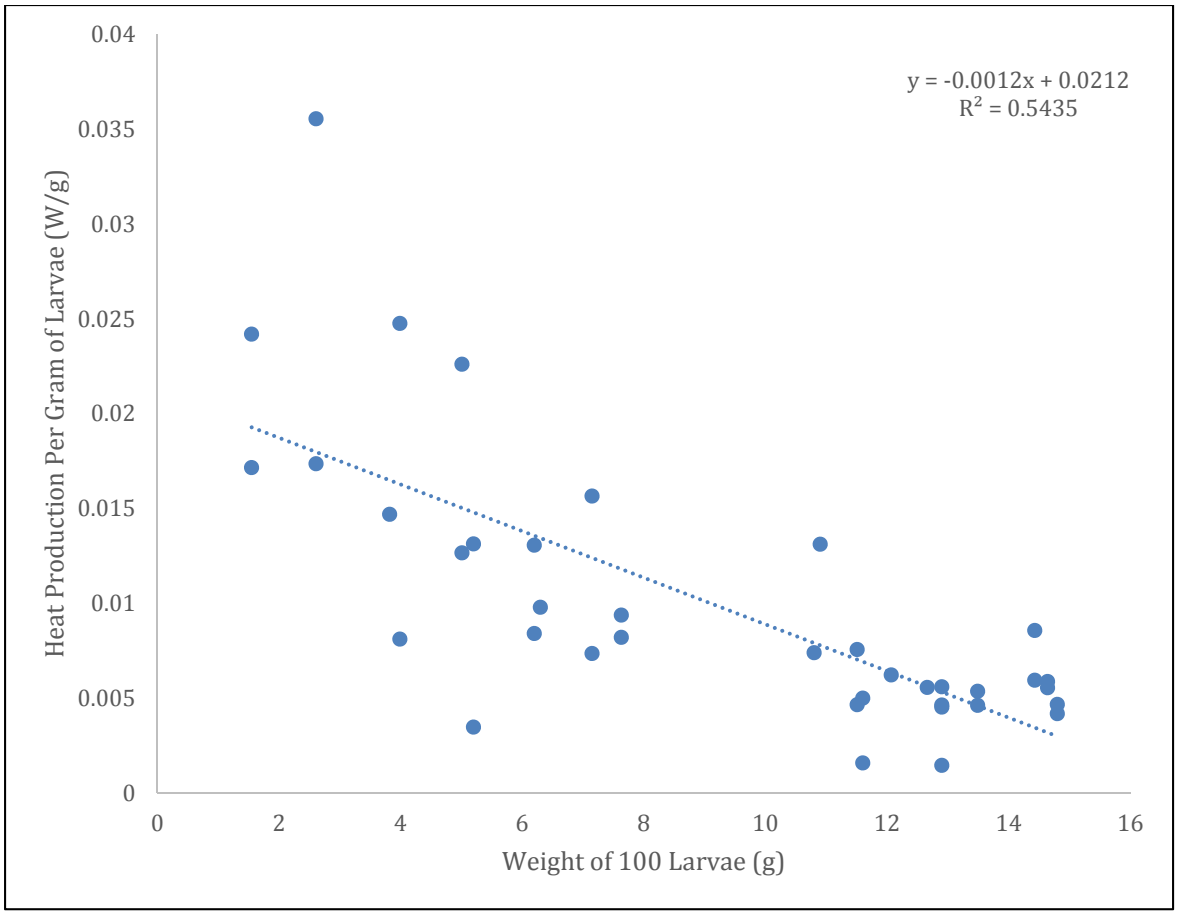

Figure 21. The heat produced per gram of larvae decreases with the weight of the larvae

Heat Production per Individual and Per Gram

The heat generated per individual larva and on a per weight basis was significantly different in different sized groups of larvae. For example, throughout their lifecycle, larvae within groups of 300 produced an average (± one standard deviation) of 0.000667 (±0.000136) W of heat per individual, whereas those in groups of 500 produced and average of 0.000494 (±0.000202) W. Larvae in groups of 100 produced significantly more heat per individual larva than the other two groups, as shown in Figure 22. Similarly, on a per gram basis, groups of 300 larvae produced an average of 0.0102 (±0.0056) W/g throughout their lifecycle, while groups of 500 produced an average of 0.00575 (±0.0037) W/g. P-values from the

SAS Glimmix function, which demonstrate these significant differences are shown in Tables 4 and 5.

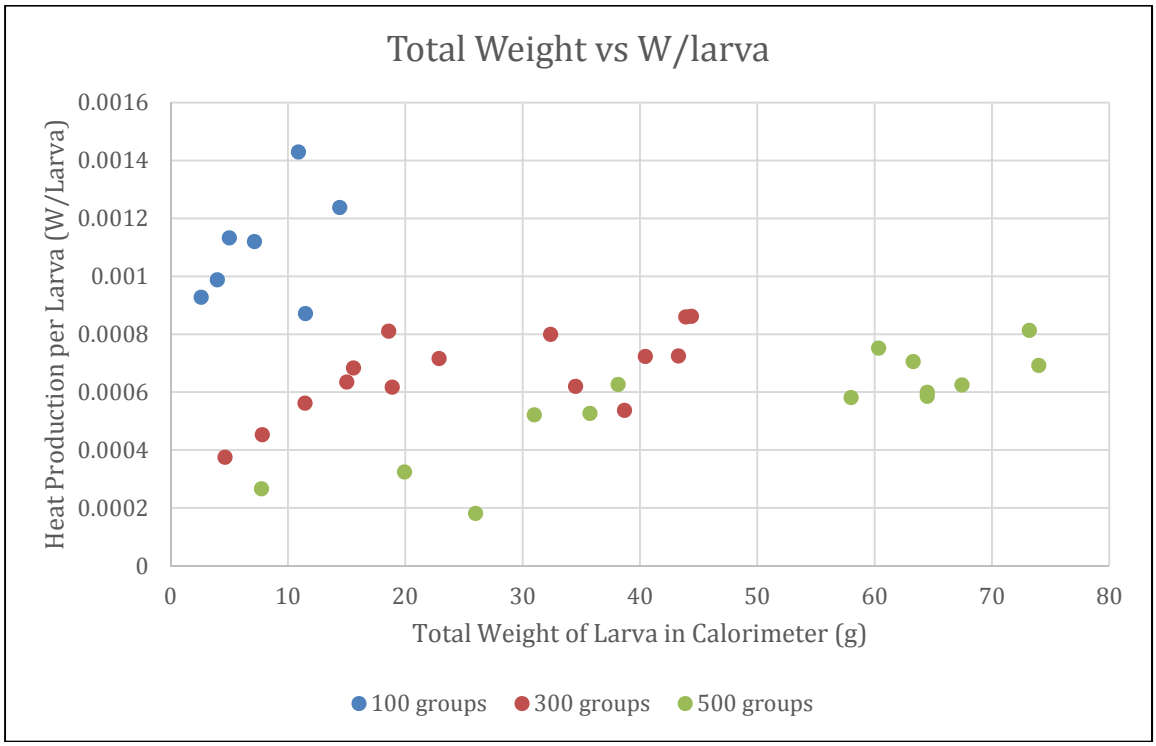

Figure 22. Heat production per individual larva was greater in larva within groups of 100 and lower in groups of 300 and 500.

Table 4. The average lifetime heat production per individual larvae in a given group size and the significant p-values between different group sizes.

		P-Values from T-test		
		Group Size		
Group Size	Average Heat Per Individual Over Lifespan (W) [μ±SD]	100	300	500
100	0.00106±0.00030	-----	0.00014	<0.0001
300	0.00067±0.00014	0.00014	-----	0.00772
500	0.00049±0.00020	<0.0001	0.00772	-----

Table 5. The average lifetime heat production per gram of larvae in a given group size and the significant p-values between different group sizes.

		P-Values from T-test		
		Group Size		
Group Size	Average Heat Per Gram Over Lifespan (W) [μ±SD]	100	300	500
100	0.01826±0.00010	-----	0.00193	0.0334
300	0.01023±0.00565	0.00193	-----	0.01353
500	0.00575±0.00371	0.03338	0.01353	-----

The differences in heat production per individual and on a per gram basis differed significantly between the group sizes, despite the fact that the different sized groups were being kept at the same stocking density of 2.5 larva per cm^2. Figures 23 and 24 again demonstrate that heat production on a per larva basis decreases as the size of the groups increase. These two figures also make it clear that this trend is not affected by the age, or by extension the weight, of the larvae, because the trend occurs in young and old larvae. This was not the expected outcome, as it was expected that if the larvae were maintained at the same stocking density then they would produce the same amount of heat per individual and per gram, regardless of the size of the group.

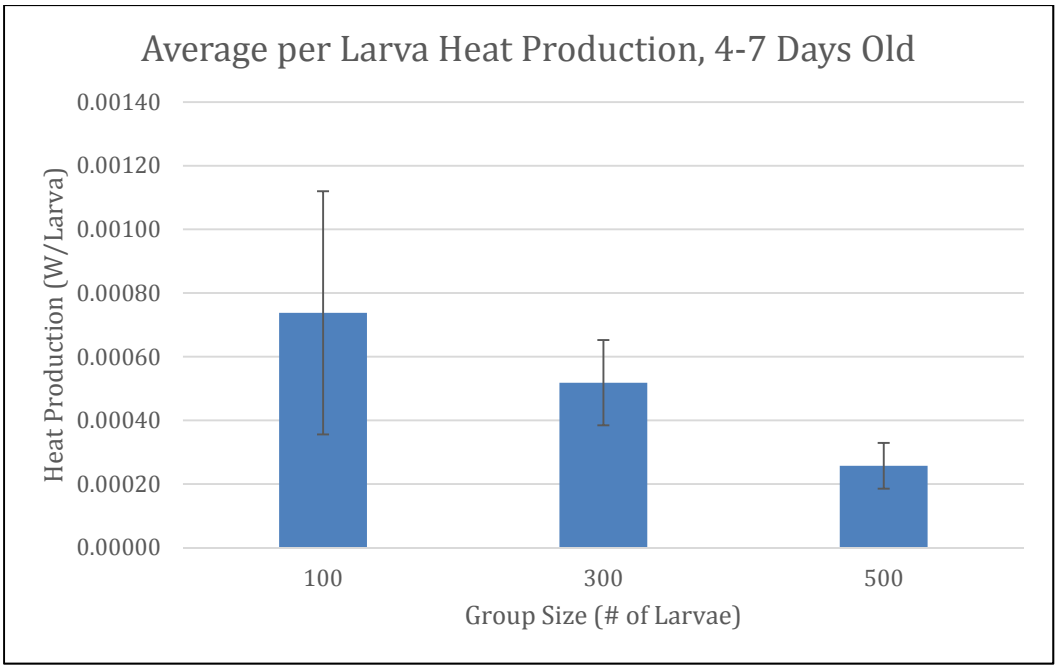

Figure 23. The amount of heat produced per individual larva decreases as the size of the group increases in young, light larvae.

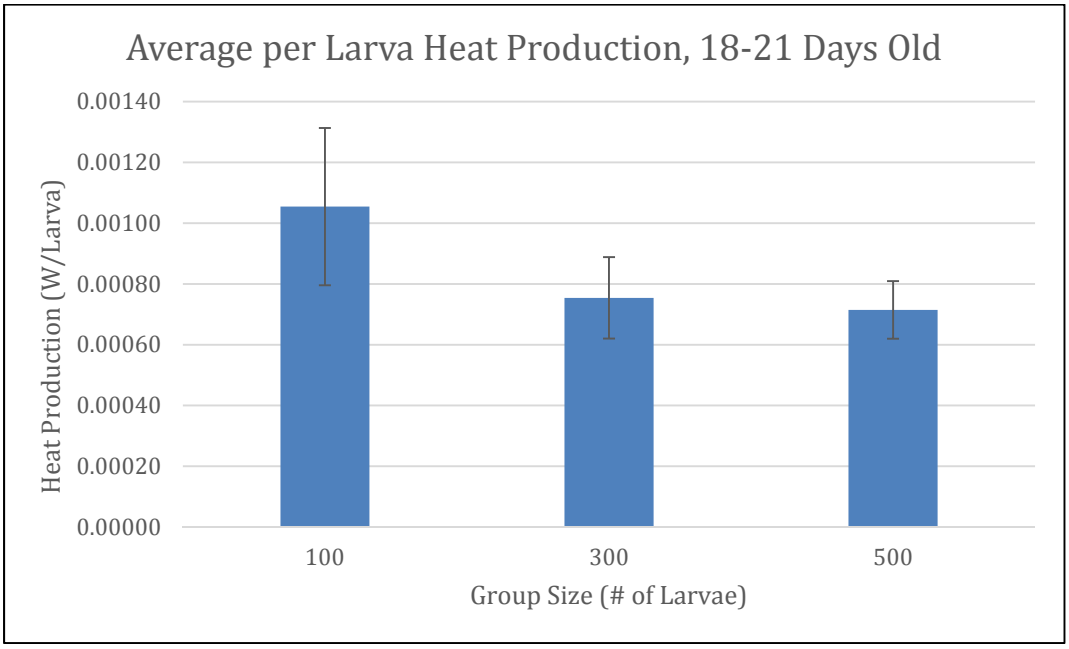

Figure 24. The amount of heat produced per individual larva decreases as the size of the group increases in old, heavy larvae.

Ammonia Production

The ammonia sensor attached to the calorimeter while the larvae were contained inside, with fresh feed, did not yield any detectable ammonia measurements. Therefore, it did not appear that the larvae were emitting any ammonia gas directly as waste. (The ammonia sensor was sensitive to ammonia concentrations as low as 2 ppm.) However two analysis of BSFL frass, conducted by Waypoint Analytical, yielded ammonia results.

The first analysis found that that BFSL frass, in its original state, before drying, was made up of 0.24% ammoniacal-nitrogen. The second analysis found that ammoniacal-nitrogen made up 0.39% of the frass. As a result, the weight of ammonia that could have been produced from the first and second analyses were 2.18 kg and 3.53 kg per metric ton (4.80 lbs and 7.79 lbs per short ton) of frass, respectively.

Conclusions

This study found that the heat production of black soldier fly larvae is significantly correlated with the age and size of the larvae. The total amount of heat produced by a group of BSFL is higher for older, larger larvae, than it is for young and small larvae. This conclusion makes sense intuitively as older, larger larvae have larger bodies and should thus produce more total heat per individual, which this study found to be true. The study also found that as the larvae grow, they produce less heat per gram of body weight, which was also expected. This study also confirmed that the larger the larval group size is, the greater the amount of total heat produced.

However, the average amount of heat produced per larvae and per gram differed significantly between different sized test groups, even when the groups were of the same size and weight. This result was unexpected because each size test group was tested in a holding container that produced equivalent population densities of 2.5 larvae per cm^2. Consequently, it was expected that the heat produced per individual and per gram of larvae would be the same despite the different group sizes, as long as the stocking density remained constant. Had this been the case, this study could have concluded that BSFL would produce a known amount of heat per individual and per gram when held at a specific population density. The result of this conclusion would have allowed for a projection of how much heat a much larger group of BSFL would have produced in a rearing facility, assuming they were held in a containers resulting in the same population density. Conversely, since the larvae from different size groups did not produce the same heat per individual or per gram, this study cannot conclude that BSFL will always produce heat at the same rate, even if they are at the same population density.

In regards to ammonia production, this study determined that any ammonia produced from a population of BSFL is not released directly by the larvae, but rather is released from their frass. The study found the amount of ammoniacal-nitrogen produced from BSFL frass to be between 4.80 and 7.79 lbs per ton of frass. It is worth noting that, since this study had no way of separating BSFL frass from old

chicken feed, it is not clear how much of this ammoniacal-nitrogen actually came from the frass versus what was produced naturally by leftover feed. However, this data can still be used to estimate how much ammonia would be produced from a large mix of BSFL frass and leftover feed.

Discussion

Uses for this Study's Data

BSFL in this study did not produce a consistent amount of heat per individual and per gram throughout the testing. Using this heat production rate would have been the most accurate method for estimating total heat production from BSFL within a facility. However, even though it is not the most accurate or the ideal method to estimate heat production, extrapolating the data from this study still allows us to estimate how much heating and ventilation would be needed to maintain optimal growing conditions in a BSFL rearing facility. For example, if BSFL within a facility were being reared in containers holding aggregates of 5,000 BSFL at population densities of 2.5 larvae per cm^2, the maximum amount of heat produced by the entire population of BSFL could be estimated by multiplying the maximum heat production of a group of 500 larvae (0.407 W) by the number of groups of 500 larvae stored in the container. In this example, there are 10 groups of 500 present:

$$\left(\frac{5{,}000}{500}\right) * 0.407W = 4.07W$$

Therefore, using this method, we would estimate that a group of 5,000 larvae would produce a heat rate of 4.07 W. Applying this method to the entire population in a facility, we can estimate the facility's entire BSFL heat production, instead of one container. This result yields the maximum heat production the entire population would produce.

Additionally, the total amount of ammonia produced could be conservatively estimated by multiplying the maximum amount of ammonia produced in this study – 7.79 lbs per ton of frass – by the estimated amount of frass left over from the total population. These conservative values for maximum heat and ammonia production allow for the determination of the maximum cooling, heating, and ventilation requirements needed to keep the space at ideal conditions of 27° C and 65% RH year-round in a given location. To be conservative, it is recommended that, for the winter, the engineer assume zero heat production by the BSFL when considering heating needs. Thus the heating design would assume warming an empty room to

the target conditions during the wintertime with no assistance from internal heat production.

Using this data to design BSFL rearing facilities could allow livestock producers to construct energy efficient accommodations for BSFL at their livestock facilities. As a result, BSFL could be used as a resource to manage the waste produced by livestock at the facility and could furthermore be used as a renewable, local, and easily accessible feed source for the livestock, whether it be poultry, swine, cattle, or fish production.

Unexpected Findings

This study found that BSFL produced significantly more heat per individual larva, and per gram, when in groups of 100 than they did in groups of 300 and 500. Since the stocking density was kept constant at 2.5 larva per cm^2 throughout all the groups, it was expected that heat production would be the same on a per individual and per gram basis in all the groups. It is not clear why larvae from the smallest groups yielded the most heat per individual and per gram. It was suggested that perhaps there is some type of behavioral change in larvae of larger groups, which causes the larvae to produce less heat. Behavioral changes could potentially include the shape the aggregate forms while in the calorimeter or changes in the amount of movement.

In the former case, the shape that the aggregate takes as a whole could affect the amount of heat that is dissipated throughout the calorimeter. It was assumed that larvae would spread evenly across the bottom of their holding container while in the calorimeter. However, if the larvae instead gathered into a mass, for example the shape of a ball, then the amount of heat dissipated from the total group may be affected by the reduction in surface area to mass and the insulative properties of the larvae. In this case, a smaller group of 100 larvae would have a larger surface-area-to-mass ratio, and would thus release heat more effectively to the environment. Larger groups, on the other hand would have a lower surface-area-to-mass ratio, and as a result would likely be more insulated and not release heat as effectively to the surrounding air.

In the latter case, it was suggested that perhaps a drop in oxygen availability within the chamber, or temperature rise above ideal conditions, could have led to a reduction in activity amongst the BSFL inside. Since the calorimeter and air ports were sealed during measurements in this study, there was very little, if any, fresh air moving through the holding space. As a result, it is possible that the larvae – particularly the larger groups of older and heavier larvae – consumed enough oxygen that reduced oxygen availability and increased in carbon dioxide in the space caused the larvae to change their behavior, i.e. a reduction in movement and food consumption. Similarly, the lack of fresh air ventilation means that the temperature inside the calorimeter increased as well during measurements. Therefore, it is possible that the temperature on the inside of the calorimeter became hot enough, particularly for larger groups that were producing more heat, that that the BSFL became stressed and reduced movement or changed their behavior in another manner which reduced heat production.

However, it was not possible to see the larvae while they were being measured in the calorimeter during this study. There were also no devices in place to measure the oxygen concentration, carbon dioxide concentration, or the temperature of the air inside the calorimeter. Consequently, this study provides no observational evidence to support either of the above theories as to why the heat production per individual and per gram dropped significantly as the group size of the larvae increased.

Moreover, this study found an interesting trend in the total amount of heat produced by each group size. Since it was hypothesized that consistent stocking densities would yield consistent heat production by individual larvae of the same age and weight regardless of the group size, the study expected to see an increase in total group heat production that coincided with increase in group size. For example, if all of the larvae had been producing the same heat per individual, then the total amount of heat produced by a group of 500 larvae should have been five times the amount of heat produced by a group of 100, assuming they group were at the same age and weight per 100 larvae. This was not the result yielded in the study, as the highest heat production rate observed for an older group of 500 was 0.407 W, while

the highest heat production produced by a group of 100 at the same size and age was about 0.12 W (Figures 12, 14 , 17, and 19). Thus despite a fivefold increase in the amount of larvae in the calorimeter, the group heat produced by the 500 group was only 3.4 times the heat produced by the 100 group. This unexpected result was most likely due to the factor which caused the heat rate per individual and per gram to differ between group sizes, although, as stated above, the cause for these trends is not known.

Recommendations for Future Work

Considering the unexpected results discussion above, we would suggest that future studies take the time to observe BSFL behavior outside of ideal conditions, such as at temperatures well above 27°C and in containers with low oxygen, high carbon dioxide concentrations. Noting how the larvae's behavior changes as the surrounding temperature rises or as oxygen levels decrease may lead to important findings as to why heat production in different sized groups is not consistent. These observations could also provide important information for people trying to raise BSFL for commercial application. For example, if it was determined that BSFL change their behavior, heat production, food consumption, or growth rates at high temperatures or low oxygen levels, then when designing a rearing facility it becomes imperative to make sure the facility is designed such that all of the rearing containers in the facility receive adequate ventilation to keep the BSFL in each container in ideal conditions. By extension, if a calorimeter-based study similar to this one is to be replicated, we suggest putting devices in place to measure the air temperature and carbon dioxide levels inside the calorimeter while taking data measurements, to perhaps gauge whether or not the BSFL's behavior is changing while in the calorimeter, using prior behavioral observation as a reference.

We also recommend observing the movement patterns of BSFL – in particular, do they spread out evenly across a space or bunch up together. The size, shape, and surface area of the aggregate as a whole may play a factor in how well heat is disperse from the aggregation. So it would be beneficial to know what type of shape the aggregation takes while inside the calorimeter. It may also be interesting

to measure the average surface area of individual BSFL throughout their growth and correlating individuals' surface area to heat production. This would be alike to how heat production rates are considered in larger animals, such as humans, although in terms of commercial application measuring the surface area of BSFL is probably less practical than measuring weight or knowing the number of larvae.

Finally, in regards to the calorimeter itself. We suggest that anyone attempting to build a gradient calorimeter similar to the one described here use solid thermocouple wires when constructing the thermopile assembly. In this study, stranded thermocouple wire was used. While stranded wire was more flexible and easier to twist into junctions than solid wire, it also proved to be much more fragile and easily broken and torn. As a result, a lot of time was spent repairing and replacing thermocouple junctions where wires had broken or come apart. Using solid wire would likely reduce the likelihood of junctions coming apart once put together and reduce the chances of wire breaking.

Appendix A. Additional Calibration Results

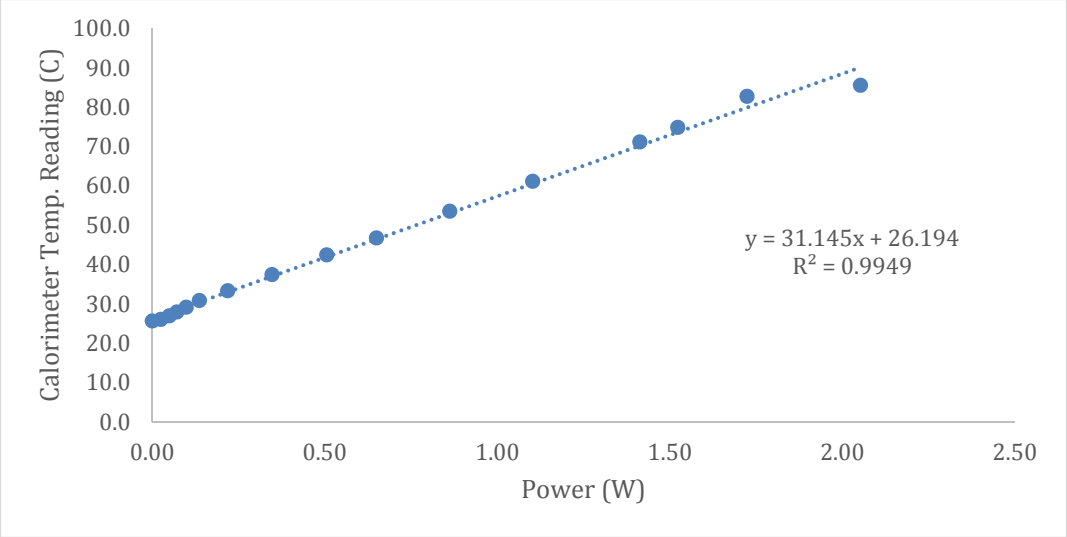

Figure A.1. The calibration curve and equation between heat production rate and temperature readout from calorimeter calibration with one airflow port open to the outside air and the other attached to an ammonia sensor.

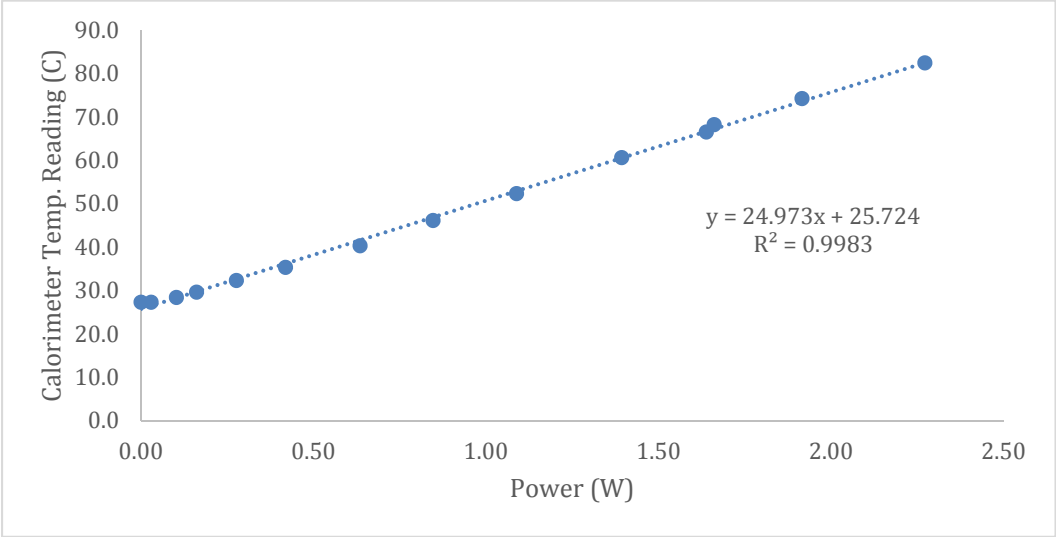

Figure A.2. The calibration curve and equation between heat production rate and temperature readout from calorimeter calibration with both airflow ports completely closed.

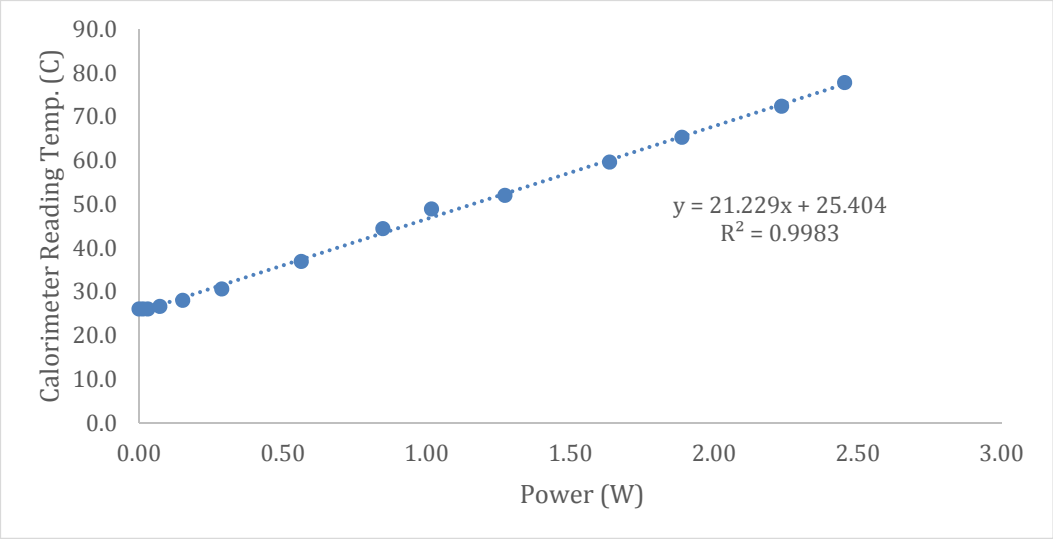

Figure A.3. The calibration curve and equation between heat production rate and temperature readout from calorimeter calibration with one airflow port receiving forced fresh air at a rate of 18.5 air exchanges per hour and the other attached to an ammonia sensor.

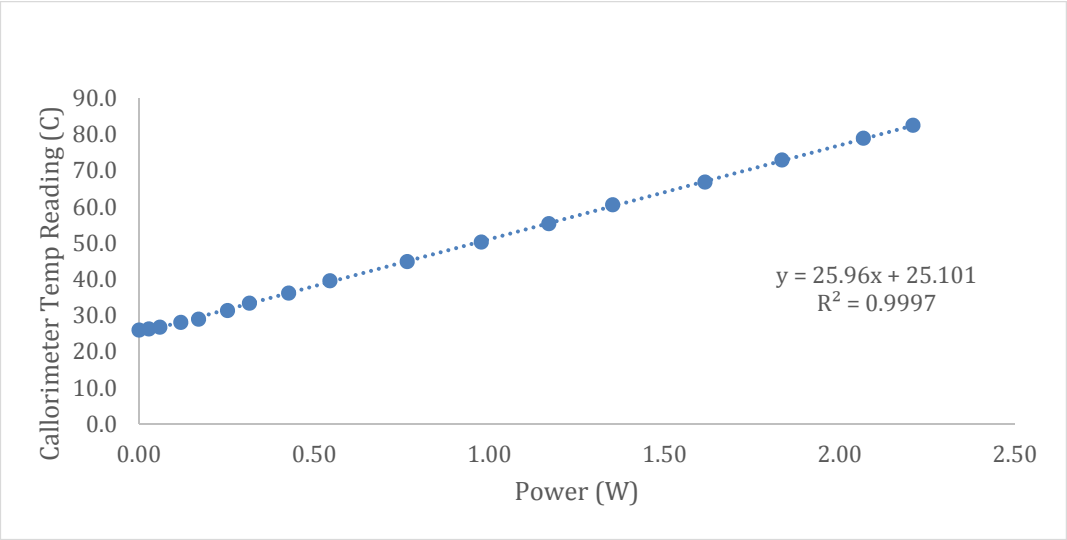

Figure A.4. The calibration curve and equation between heat production rate and temperature readout from calorimeter calibration with one airflow port receiving forced fresh air at a rate of 1.65 air exchanges per hour and the other attached to an ammonia sensor.

Appendix B. Standard Operating Procedures for BSFL Daily Care

Feeding

1. Measure out 300g of water using a plastic cup and analytical balance
2. Measure out 200g of chicken feed using DRY cup on analytical balance
3. Add feed and water to mixing bowl and mix together to form a consistent mash
4. Remove % feed from corresponding to aggregation size for that day and place in appropriate testing tray
    - If 100 larvae testing → remove of feed (10 g)
    - If 300 larvae testing → remove of feed (30 g)
    - If 500 larvae testing → remove of feed (50 g)
5. Place all food not removed for testing sample into main population container into main larvae mass
6. **Clean out mixing bowl so no contaminants remain for the next day**!

Calorimetry

1. Unscrew top of calorimeter and gently remove top
2. Gently count out the number of larvae being tested that day
    - Count out before feeding commences
3. Place testing population into the appropriate testing tray
    - If 100 larvae testing → 2.5-in x 2.5-in tray
    - If 300 larvae testing → 5.75-in x 3.25-in tray
    - If 500 larvae testing → 5.75-in x 5-in tray
4. Place appropriate food allocation for test population into the tray, into the aggregate (See above)
5. Place tray with food and larvae into calorimeter onto rubber stoppers
6. Run wires from top thermopile through insulation
7. Gently place top onto calorimeter frame and screw into place
8. Plug in thermocouple reader
9. Record data for 2.5 hours

10. After 2.5 hour run time, stop data collect, remove and turnoff thermocouple reader
11. Unscrew top of calorimeter and carefully remove – **DO NOT DAMAGE WIRING!**
12. Remove larvae and tray from calorimeter and place back into primary container with the rest of the population

Other Daily Tasks

1. Check that environmental chamber temperature and humidity are within acceptable ranges
    - Temp. = 27° C ± 0.3° C
    - Humidity = 60% ± 3%

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